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**THE ECOLOGY AND CONTROL OF CUTANEOUS LEISHMANIASIS IN THE
SUB-ANDEAN REGION OF SOUTH-WEST COLOMBIA**

Thesis submitted for the degree of Doctor of Philosophy in the University of London

By

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DEDICATION

To my father who transmitted to me the passion for entomology. To my mother who has given to me the example of strength and constancy in life. To my wife Stella for her love and support throughout the journey of this PhD; and to my daughters Andrea and Laura for their love and understanding.

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ABSTRACT

This thesis describes a series of studies designed to improve our understanding of the transmission cycle of cutaneous leishmaniasis (CL) in the coffee-growing sub-Andean region of Huila department and to explore the use of insecticide treated bednets as an alternative control measure to the current policy of house spraying. The thesis is divided into six chapters, including four results chapters. **Chapter 1** reviews the public health importance of CL in the Andean region, in Colombia and in Huila department; explores the possible risk of CL in coffee plantations at regional, departmental and municipal levels; and presents a brief summary on control strategies in the Andes, Colombia and Huila. **Chapter 2** describes an exploratory study in seven representative areas within the sub-Andean region (1000 - 2000 m a.s.l.) of Huila department designed to identify possible CL vectors and the ecological determinants for their distribution and abundance. The study helps to: (i) explain the current distribution of CL in Huila department; (ii) identify the boundaries of the epidemic area; and (iii) identify new areas of potential risk for the disease which should be considered for monitoring or prevention programs. The main findings were: (1) the CL foci of Huila was identified geographically; (2) *Lutzomyia longiflocosa* appears to be the principal sandfly vector, having a narrow ecological niche defined largely by altitude, temperature and a preference for a well structured forest or forest-like habitat (i.e. traditional coffee-growing area); (3) there was no evidence for complete adaptation of *L. longiflocosa* to intensive coffee plantations; and (4) *L. nuneztovari* is a generalist species which has at most a limited secondary vectorial role in this region. **Chapter 3** describes a cross-sectional study at household level in three villages (267 houses) designed to (i) identify environmental risk factors for the suspected vectors and (ii) identify demographic, environmental and entomological risk factors for disease. The main findings were: (1) stronger evidence incriminating *L. longiflocosa* as the main vector, and confirming the less important role of *L. nuneztovari*, (2) the detected risks confirmed the feasibility of the use of insecticide treated bednets (ITNs) as a control measure for CL. **Chapter 4** describes a series of field studies to evaluate the use of lamdacyhalothrin treated bed nets as an alternative control measure (to house spraying) for CL within the study area: (1) the entomological efficacy of ITNs was tested under controlled conditions; (2) the entomological effectiveness (measured indirectly by indoor CDC light traps) of ITNs and house spraying were both measured in a

household-based intervention trial; (3) the reliability of light traps as an indicator of indoor sandfly exposure (in the previous study) was tested by comparison with indoor human landing catches; and (4) field bioassays were used to measure the residual lethal effect of the insecticide up to 4 months after both interventions were implemented in the effectiveness trial. Together, the efficacy and effectiveness studies showed that ITNs reduce *L. longiflocosa* indoor human landing rates, blood feeding success, and Human Blood Index. The effects of house spraying were unclear, as the reduction in sandfly numbers (fed and unfeds) observed in light traps in sprayed houses was not reflected by any reduction in human landing catches. **Chapter 5** describes a questionnaire study of the inhabitants in the epidemic area to evaluate their knowledge, attitudes and practices in relation to sandfly and CL control in Huila. The study showed that (i) bednets were widely used, but less so amongst the poorest households, and (ii) nets were commonly used to reduce sandfly nuisance rather than reduce the risk of CL. However knowledge of sandfly involvement in CL transmission was positively associated with net usage. This information should help inform the design of future ITN campaigns in the region. Finally, **Chapter 6** summarises and integrates the main findings of the four results chapters, recommends the provision of ITNs to replace house spraying for CL control in Huila, and proposes future studies which should be prioritised.

ABBREVIATIONS

ACL	anthroponotic cutaneous leishmaniasis
AT	direct aspiration of resting sandflies from tree trunks
bh-PM	premontane moist forest
bmh-PM	premontane wet forest
bs-PM	premontane dry forest
ca.	circa, approximately
CIDEIM	Centro Internacional de Investigaciones Médicas
C.I.	confidence interval
CL	cutaneous leishmaniasis
CS	capsulated or microencapsulated suspension
DANE	Departamento Administrativo Nacional de Estadística
df	degrees of freedom
EC	emulsiable concentration
$F_{(n1, n2)}$	F test with n_1 and n_2 degrees of freedom
f/LT/n	females / CDC light trap / night
f/p/3h	females / person / 3 h
f/p/40 min	females / person / 40 min
fem.	females
GLIM	generalized linear interactive models
GM	Williams' geometric mean = $e^{\bar{x}} - 1$, where $\bar{x} = \frac{\sum \ln(x+1)}{n}$
<p>On some occasions (indicated in the text or tables, when numbers were > 0) the calculus corresponded to the geometric mean = $e^{\bar{x}}$, where</p> $\bar{x} = \frac{\sum \ln(x)}{n}$	
h	hour
ha	hectare
HBI	Human blood index (the proportion of human blood)
HL	human landing
HLC	catches by human landing
a.i.	active ingredient

inh	inhabitants
IDEAM	Instituto de Meteorología, Hidrología y Adecuación de Tierras
INS	Instituto Nacional de Salud, Colombia (National Health Institute)
ITN	insecticide treated net(s)
KAP	knowledge, attitudes, and practice
KOH	hydroxide of potassium
LD ₉₅	required dose to kill 95% of tested animal species
LRT	likelihood ratio test
LT	CDC light trap
LTC	catches by CDC light traps
m a.s.l.	meters above the sea level
m	meters
mm	millimetres
m / s	meters / second
MAM	Minimum adequate model
Max	maximum value
Min	minimum value
MCL	mucocutaneous leishmaniasis
min	minutes
n	sample size
N	North
NHS	Neiva Health Service
No.	Number
µl	microlitres
MOH	Ministerio de la Protección Social, Colombia (former Ministry of Health)
q_{25}	quantil 25 %
q_{75}	quantil 75 %
r^2	coefficient of determination for linear and nonlinear models
S	South
S.E.	standard error
SD	standard deviation
s/LT/n	sandflies / CDC light trap / night
s/p/3h	sandflies / person / 3 h
s/p/h	sandflies / person / h

s/p/n	sandflies / person / night
SSDH	Secretaria de Salud departamental del Huila (Huila Health Service)
TWINSpan	Two Ways Indicator Species Analysis
UPGMA	Un-weighted pair-group method using arithmetic averages
VL	visceral leishmaniasis
WHO	World Health Organization
WP	wettable powder formulation
χ^2	chi square test statistic
$\chi^2_{(v)}$	chi square with v degrees of freedom, as a result of applying χ^2 test
ZCL	zoonotic cutaneous leishmaniasis
95% C.I.	95% confidence interval

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1 INTRODUCTION

1.1 INTRODUCTION TO CUTANEOUS LEISHMANIASES IN THE ANDEAN REGION

The leishmaniasis are a group of diseases caused by parasites of the genus *Leishmania* and transmitted to humans by the bite of an infected female sandfly (of the genus *Lutzomyia* in the New World and *Phlebotomus* in the Old World) (Desjeux 1992). The most common clinical form is cutaneous leishmaniasis (CL) with an annual global incidence of 1 to 1.5 million cases (WHO 2002).

CL in the New World is a disease with zoonotic characteristics. It involves at least 13 species of the genus *Leishmania*, 32 proven or suspected vectors species and around 40 mammal species as reservoirs. This implies the existence of many transmission cycles, thus showing a complex epidemiology (Grimaldi and Tesh 1993).

Once considered as a disease associated with intrusion of humans into undisturbed forested areas, there is now overwhelming evidence that CL transmission in the New World can occur within the domestic environment, specially in new human settlements (where herbaceous and bush strata in the forest has been replaced with crops which need shade) (Le Pont and Pajot 1981; Aguilar *et al.*, 1989; Lainson 1989). These modifications in the transmission cycle led to new approaches to control, allowing the possibility for control measures such as house spraying (Campbell *et al.*, 2001).

In the Cordillera (mountain chains) de los Andes, CL is distributed from about 10° N to approximately 20° S, including, from north to south, the following countries: Venezuela, Colombia, Ecuador, Peru and Bolivia. No CL cases have been reported in the Andes of Chile and Argentina. CL cases have a wide distribution within the Andean countries, being reported in 89% (94 / 106) of the departments of all the five countries. The mean number of reported cases of leishmaniasis between 1996 to 1998 was 14,082

cases, Colombia having the highest number, 6,155 cases; and Bolivia the highest incidence rate (Davies *et al.*, 2000b).

The complexity of CL epidemiology in the New World perhaps reaches its highest level in the Andes, where, until now, seven species of parasites, 13 confirmed *Lutzomyia* species as proven vectors (many of which are endemic) and around 24 species of infected mammals (possible reservoirs) have been identified (Table 1.1).

It seems that *Le. braziliensis* is responsible for the greatest number of CL cases (49%) and is the species with the widest distribution (isolated in 50 of 74 departments), followed by *Le. panamensis*, 27% of reported cases (isolated in 27 departments) (Davies *et al.*, 2000b).

The forest, the habitat considered to be of highest risk for the transmission of CL is highly diverse in the Andes and requires special attention. The limited information available on this subject allows only a partial description of the types of habitat for some of the vectors of CL. With few exceptions, where a floristic or structural-physiognomic features description were reported (Alexander *et al.*, 1995d; Valenta 1999), habitats and climatic conditions have been described roughly using the Holdridge system for classification of life zones. This system is based on three indicators: biotemperature (temperatures above freezing), mean annual rainfall and the potential evapotranspiration ratio, as determinants of climax vegetation. The broadly defined life zones are further divided into associations on the basis of local environmental conditions. Holdridge life zones have been described for transmission foci within the Andean region in the following localities (see Figure 1.1) divided according to altitudinal ranges:

a) Foothills region (400 - 1000 m a.s.l.). In Landazuri, Colombia (vectors: *L. trapidoi* and *L. gomezi*), and Alto Beni, Bolivia (vectors: *L. llanosmartinsi* and *L. yucumensis*), the habitat was tropical rain forest (Muñoz-Mantilla 1998) (Alcais *et al.*, 1997). Nevertheless differences could be expected between these forests because the Bolivian forest is a transition to the Amazon rain forest, while the Colombian forest belongs to a transition to the lowland forest of the mid Magdalena valley. In Arboledas, Colombia

Table 1.1 Confirmed parasites, vectors and *Leishmania* infections in non-human mammals for cutaneous leishmaniasis in the Andes (Taken from Davies *et al.*, 2000). Vectors which were not likely to be present in the Andean region, > 400 m a.s.l. in the Andes, were excluded.

Parasite		Vector		Infected mammal	
<i>Leishmania</i> species	Distribution within region	<i>Lutzomyia</i> species	Distribution within region ^a	Species	Country isolation ^a
<i>Le. amazonensis</i>	B,C,E,P,V	<i>L. nuneztovari</i>	B,C,P,V	<i>Akodon</i> sp. (Rodentia)	B
				<i>Conepatus chinga rex</i> (Carnivora)	B
				<i>Oligoryzomys</i> sp. (Rodentia)	B
				<i>Potos flavus</i> (Carnivora)	E
				<i>Sciuris vulgaris</i> (Rodentia)	B
				<i>Tamandua tetradactyla</i> (Edentata)	E
<i>Le. braziliensis</i>	B,C,E,P,V	<i>L. carrerai</i>	B,C,E,P,V	<i>Canis familiaris</i> (Carnivora)	C,P,V
		<i>L. gomezi</i>	C,E,P,V	<i>Cerdocyon thous</i> (Carnivora)	V
		<i>L. llanosmartinsi</i>	B,P	<i>Co. chinga rex</i>	B
		<i>L. ovallesi</i>	C,V	<i>Equus asinus</i> (Perissodactyla)	C,V
		<i>L. spinicrassa</i>	C,V	<i>Oryzomys concolor</i> (Rodentia)	V
		<i>L. yucumensis</i>	B,P	<i>Rattus rattus</i> (Rodentia)	E
<i>Le. colombiensis</i>	C	<i>L. hartmanni</i>	C,E	<i>Zygodontomys microtynus</i> (Rodentia)	V
				<i>C. familiaris</i>	C
<i>Le. mexicana</i>	C,E,V	<i>L. ayacuchensis</i>	E,P	<i>Choloepus hoffmani</i> (Edentata)	E
		<i>L. ovallesi</i>		<i>C. familiaris</i>	E
<i>Le. panamensis</i>	C,E	<i>L. panamensis</i>	C,V	<i>C. familiaris</i>	E,C
		<i>L. trapidoi</i>	C,E	<i>Bradypus griseus</i> (Edentata)	C
				<i>Ch. hoffmani</i>	C
				<i>Heteromys dermarestianus</i> (Rodentia)	C
<i>Le. peruviana</i>	P	<i>L. ayacuchensis</i>		<i>C. familiaris</i>	P
		<i>L. peruensis</i>	P	<i>Didelphis albiventris</i> (Marsupiala)	P
		<i>L. verrucarum</i>	P,V	<i>Phyllotis andinum</i> (Rodentia)	P
<i>Le. venezuelensis</i>	V			<i>Felix domesticus</i> (Carnivora)	V
<i>Le. braziliensis</i> or <i>Le. panamensis</i>				<i>Melanomys caliginosus</i> (Rodentia)	C
				<i>D. marsupialis</i>	C
				<i>Microzomys minutus</i> (Rodentia)	C
				<i>Mocoereus demerarae</i> (Marsupiala)	C
				<i>R. rattus</i>	C
				<i>Sylvilagus braziliensis</i> (Lagomorpha)	C
<i>Le. peruviana</i> or <i>Le. guyanensis</i>				<i>Akodon</i> sp. (Rodentia)	P

^a B: Bolivia, C: Colombia, E: Ecuador, P: Peru, V: Venezuela.



Figure 1.1 Some of the study foci of cutaneous leishmaniasis in the Andes. References: 1 (Scorza *et al.*, 1984); 2 (Alexander *et al.*, 1992); 3 (Muñoz-Mantilla 1998); 4 (Velez *et al.*, 1991); 5 (Cárdenas *et al.*, 1999); 6 (Alexander *et al.*, 1995d), 7 (Montoya-Lerma *et al.*, 1999); 8 (Hashigushi *et al.*, 1990); 9 and 10 (Davies *et al.*, 1997); 11 (Le Pont and Desjeux 1986; Le Pont *et al.*, 1989b); 12 (Torres *et al.*, 1998); 13 (Martinez *et al.*, 1999). Map source: www.lib.utexas.edu/maps/americas.html.

(vector: *L. spinicrassa*), the forest was classified as very humid subtropical forest (Alexander *et al.*, 1992).

b) Sub-Andean region (1000 - 2000 m a.s.l.). In Dagua and Samaniego, in Colombia (suspected vector: *L. columbiana*), the reported habitats were tropical dry forest and low montane dry forest, respectively (Montoya *et al.*, 1990; Montoya-Lerma *et al.*, 1999). In Cajuata, in Las Yungas, Bolivia (vector: *L. nuneztovari*), the forest was reported as deciduous forest with some xerophytic elements (Martinez *et al.*, 1999).

c) Andean region (> 2000 m a.s.l.). In both, Paute, Ecuador (vector: *L. ayacuchensis*), and Purisima valley, Peru (vector: *L. peruensis* and *L. verrucarum*), the main habitat was described roughly as xerophytic vegetation (low shrubs, agaves and cacti) (Hashigushi *et al.*, 1990) (Villaseca *et al.*, 1993).

In Venezuela, using data on the country-wide distribution of sandfly vectors of the verrucarum group, the main habitats for each species were identified, according to a modification of the Holdridge life zones (Feliciangeli *et al.*, 1992). *L. spinicrassa* (distributed in the foothill region) and *L. youngi* (distributed in the foothills and the sub-Andean regions) were found in lower montane moist forest and premontane moist forest, as well as in montane dry forest. *L. ovallesi* was present in seven life zones (from tropical moist forest to lower montane dry forest).

Hence, there is a wide variety of habitats in the Andes where transmission may occur. Some vector species are adapted to few habitats (i.e. endemic species like *L. columbiana*) while others have adapted to a wide variety of habitats (i.e. generalistic species like *L. ovallesi*). In many of the studies carried out in the sub-Andean region the coffee crop is mentioned as a habitat associated with sandfly vectors. In addition, it seems that transmission indoors is also very important with many proven or suspected vectors apparently endophagic, e.g. *L. youngi* in Venezuela (Scorza *et al.*, 1984), *L. spinicrassa* (Alexander *et al.*, 1992) and *L. columbiana* (Montoya-Lerma *et al.*, 1999) in Colombia, *L. ayacuchensis* in Ecuador (Hashigushi *et al.*, 1990), and *L. peruensis* and *L. verrucarum* in Peru (Llanos-Cuentas and Davies 1991).

1.2 CUTANEOUS LEISHMANIASIS IN COLOMBIA AND HUILA DEPARTMENT

The study of the different transmission cycles of CL in Colombia started during the 1980s when the obligatory reporting of cases established by the MOH showed that the disease had an important impact in the population and the "Program for the Control and Surveillance of CL" was established. The known main foci of CL began to be investigated with the aims of identifying the vectors, parasites and reservoirs involved in transmission and the risk factors for the disease in order to use this knowledge in the implementation of control programmes to be applied to other foci of the disease. Until now only a low proportion, (3 / 13) of the main foci (from a total of 78 foci) of CL detected in the 1980s (Corredor *et al.*, 1986) have been investigated in any detail: Tumaco (Weigle *et al.*, 1993; Travi *et al.*, 1988), Landazuri (Muñoz-Mantilla 1998), Arboledas (Alexander *et al.*, 1992); though some other foci have been studied superficially: including Montebello (Velez *et al.*, 1991), San Roque (Velez *et al.*, 1987), Dagua (Montoya *et al.*, 1990), La Guaira (Alexander *et al.*, 1995d), Samaniego (Montoya-Lerma *et al.*, 1999), Villeta (Pardo *et al.*, 1996).

One of the most significant recent outbreaks of CL in Colombia was in the coffee growing Andean department of Huila from 1993 to 1996. Following a series of small scale pilot studies by INS (described later), this PhD was designed to investigate this focus in detail in order to help with the development of a control strategy in the case of future outbreaks in this region.

Colombia is the third country, after Brazil and Indonesia, in the list of the twelve so-called "megadiversity" countries (Sarukhan and Dirzo 2001), where 70% of the earth's biological diversity is found. The ecological diversity is reflected in the complexity of transmission cycles of CL within Colombia. Table 1.2 lists our current knowledge on sandfly vectors and parasites. Basic references for sandfly and parasites distribution were Young & Duncan (1994) and Corredor *et al.* (1990), respectively. Species distribution was organized according to the classification in Natural Regions (IGAC Instituto Geográfico Agustín Codazzi 1998) (Figure 1.2) and the altitudinal division of the Andes (Section 1.1). Six species of *Leishmania* parasites and sixteen sandfly vectors (four proven, seven suspected species and five which are proven vectors in other countries) have been identified. About twelve mammals are suspected reservoirs

Table 1.2 Vectors and parasites of cutaneous leishmaniasis in Colombia by region.

Vector			
Region	Distribution within the region	Lutzomyia species	Leishmania species
Andina			
Cordillera Occidental			
Sub-Andean region surrounding the Patia and Cauca river basins.	Sub-Andean region surrounding the San Juan river basin.	<i>L. columbiana</i> ^b	<i>Le. amazonensis</i> , <i>Le. braziliensis</i> , <i>Le. colombiensis</i> , <i>Le. mexicana</i> , <i>Le. panamensis</i>
		<i>L. columbiana</i> ^b <i>L. lichyi</i> ^b <i>L. youngi</i> ^b	<i>Le. mexicana</i>
Cordillera Central			
Sub-Andean region surrounding the Cauca river basin.	Foothills surrounding the central part of the middle Magdalena river basin.	<i>L. gomezi</i> ^{b,c}	Feliciangeli et al., 1994 ^d ; Rodriguez et al., 1999 ^d ; Velez et al., 1991
		<i>L. trapidoi</i> ^a <i>L. gomezi</i> ^{b,c} <i>L. hartmanni</i> ^a	Velez et. al., 1987; Young et al., 1987 ^d ; Porter and DeFoliart, 1981
Sub-Andean region surrounding the central part of the upper Magdalena river basin (Tolima department).	Cordillera Oriental	<i>L. longiflocosa</i> ^b <i>L. nuneztovari</i> ^c	Cardenas et al., 1999; Martinez et al., 1999 ^d
Sub-Andean region surrounding the Catatumbo river basin.	Foothills surrounding the central part of the middle Magdalena river basin.	<i>L. spinicrassa</i> ^a	Young et al., 1987 ^d ; Arias et al., 1985 ^d , 1987 ^d
		<i>L. flaviscutellata</i> ^c	Muñoz-Mantilla, 1998 ^d
Foothills surrounding the North part of the upper Magdalena river basin.	Foothills surrounding the central part of the middle Magdalena river basin.	<i>L. trapidoi</i> ^a	Pardo et al., 1996; Feliciangeli et al., 1994 ^d ; Barrios et al., 1994 ^d
		<i>L. gomezi</i> ^{b,c} <i>L. ovallesi</i> ^{b,c}	Kreutzer et al., 1991 ^d ; Ferro C. (personal communication)
Foothills surrounding the North part of the middle Magdalena river basin in a locality of Santander department.	Sub-Andean region surrounding the upper Magdalena river basin in two localities of Huila department, several localities of Tolima and in a locality of the sub-Andean region of Santander.	<i>L. hartmanni</i> ^a	Ferro et al., 1999; Cardenas et al., 2005; Pardo et al., 2006
		<i>L. longiflocosa</i> ^b	

Table 1.2 Continued.

Vector				Reference
Region	Distribution within the region	Lutzomyia species	Leishmania species	
Sub-Andean region surrounding the central part of the middle Magdalena river basin, Bucaramanga city.		<i>L. quasitownsendi</i>	<i>Le. braziliensis</i> ?	Ferro C. (personal commur.
	Sub-Andean region surrounding the North part of the upper Magdalena river basin in a locality of Cundinamarca department.	<i>L. torvida</i> ^b	<i>le. braziliensis</i> ?	Ferro et al., 1999
Amazonia			<i>Le. amazonensis, Le. braziliensis, Le. guyanensis, Le. mexicana</i>	
Amazon lowlands.		<i>L. umbratilis</i> ^a	<i>Le. guyanensis</i> ^c	Young et al., 1987 ^d ; Le Pc 2000; Molina et al., 2000
		<i>L. carrerai</i> ^c	<i>Le. braziliensis</i>	
		<i>L. flaviscutellata</i> ^c		
		<i>L. gomezi</i> ^c		
		<i>L. yucumensis</i> ^c		
		<i>L. yuilli</i> ?		
Orinoquia		<i>L. ayrozai</i> ?		
			<i>Le. amazonensis, Le. braziliensis, Le. panamensis</i>	
	Plain next to the foothills of the cordillera Oriental.	<i>L. fairtigi</i> ^b ? <i>L. flaviscutellata</i> ^c <i>L. panamensis</i> ^c		Molina, 1999
Caribe			<i>Le. panamensis</i>	
Guajira peninsula.		<i>L. gomezi</i>		Morales et al., 1987; Ferro
		<i>L. ovallesi</i> ^c	<i>Le. panamensis</i>	
Pacifico			<i>Le. braziliensis, Le. panamensis</i>	
Alluvial valleys of Atrato and San Juan rivers. Pacific coast plain.		<i>L. trapidoi</i> ^a		Travi et al., 1988
		<i>L. trapidoi</i> ^a	<i>Le. panamensis</i>	Travi et al., 1988 ^d ; Christer
		<i>L. gomezi</i> ^c		
		<i>L. harmanni</i> ^a		
		<i>L. panamensis</i> ^c	<i>Le. panamensis</i>	

^a Proven vector; ^b Suspected vector based on epidemiological evidence, plus vectorial competence in some cases; ^c Proven vector in other country; ^d Reference for identification of parasites; unconfirmed data.

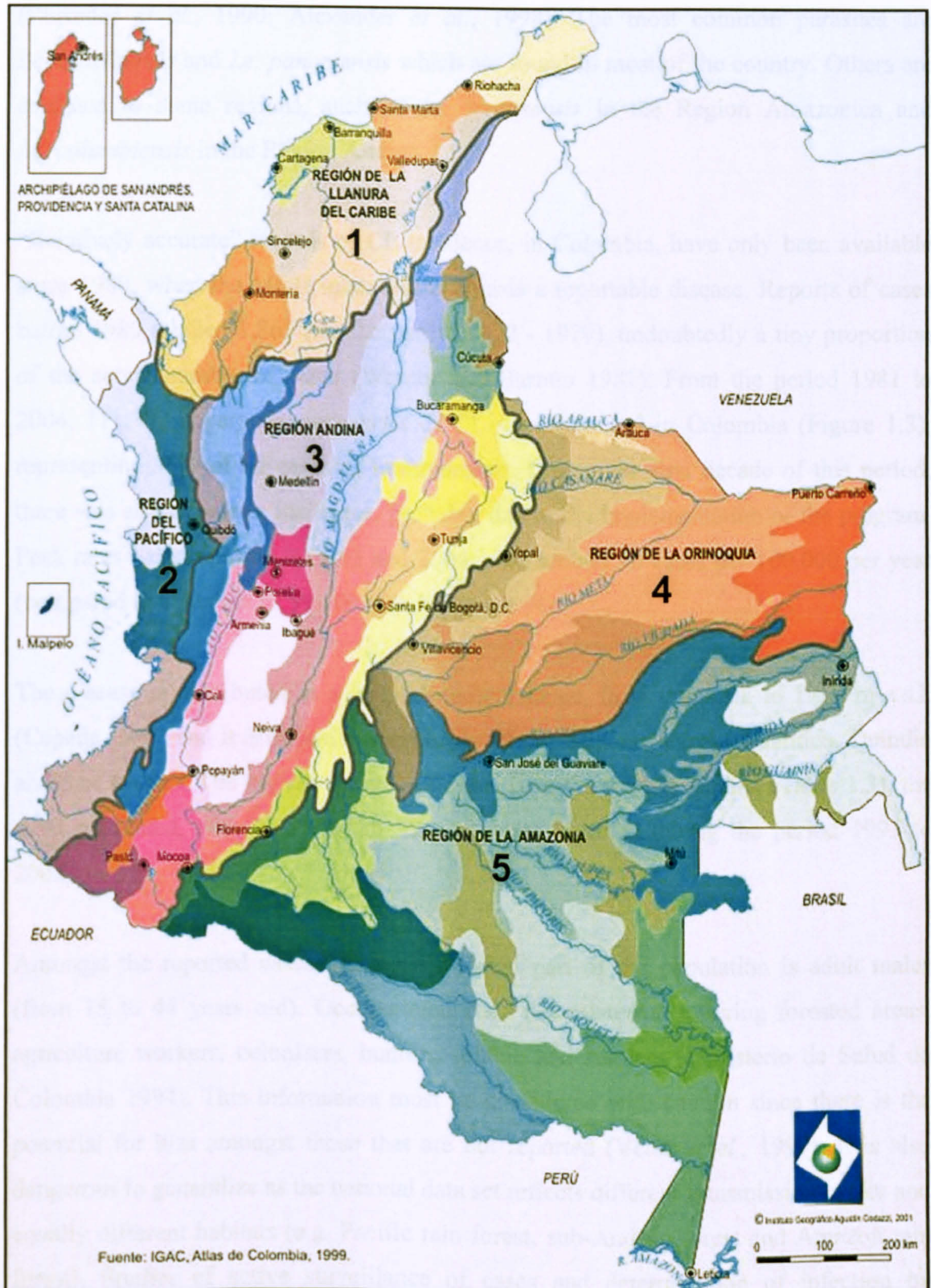


Figure 1.2 Natural Regions of Colombia: (1) Llanura del Caribe; (2) Region del Pacifico; (3) Region Andina; (4) Region de la Orinoquia; and (5) Region de la Amazonia. Each region is divided in sub-regions, 51 in total, shown in different colours. Map source: Atlas de Colombia Ver. 1.0, Instituto Geográfico Agustín Codazzi, Bogotá, 1998.

(Corredor *et al.*, 1990; Alexander *et al.*, 1998). The most common parasites are *Le. braziliensis* and *Le. panamensis* which are found in most of the country. Others are confined to some regions, such as *Le. guyanensis* in the Region Amazonica and *Le. columbiensis* in the Region Andina.

“Relatively accurate” records of CL incidence, in Colombia, have only been available since 1980, when the MOH made leishmaniasis a reportable disease. Reports of cases before 1980 totalled 1,865 (for the period 1872 - 1979), undoubtedly a tiny proportion of the actual number of cases (Werner and Barreto 1981). From the period 1981 to 2004, 111,302 apparently new cases of CL were reported in Colombia (Figure 1.3), representing 95% of all cases of leishmaniasis. During the first decade of this period, there was an increase in incidence, probably due to the implementation of the program. Peak rates were reported in 2003 and 2004, with around 90 cases per 100,000 per year (compared to a median of 59/100,000 during the 90s).

The disease is distributed in a wide altitudinal range, from 0 m a.s.l. to 1750 m a.s.l. (Cepeda 1997) and it is endemic in probably all departments, except Atlantico, Quindio and San Andres. The highest number of cases is in the Region Andina (Table 1.3), the most populated, with 72.5% of the 52,033 cases reported during the period 1993 to 2002.

Amongst the reported cases, the most affected part of the population is adult males (from 15 to 44 years old). Occupational risks are related to entering forested areas: agriculture workers, colonisers, hunters, miners and soldiers (Ministerio de Salud de Colombia 1994). This information must be considered with caution since there is the potential for bias amongst those that are not reported (Velez *et al.*, 1997). It is also dangerous to generalize as the national data set reflects different transmission cycles and equally different habitats (e.g. Pacific rain forest, sub-Andean forest and Amazon rain forest). Studies of active surveillance of cases and determination of infection by leishmanin skin tests in specific transmission areas have demonstrated the degree of variation in risk factors that can be found. In a study carried out in the tropical rain forest of Chocó, where *L. trapidoi* and *L. gomezi* (exophilic species) are the presumed vectors of *Le. panamensis*, it was shown that the most important risk factors are behavioural and micro-environmental: entering a forested area during the night, for

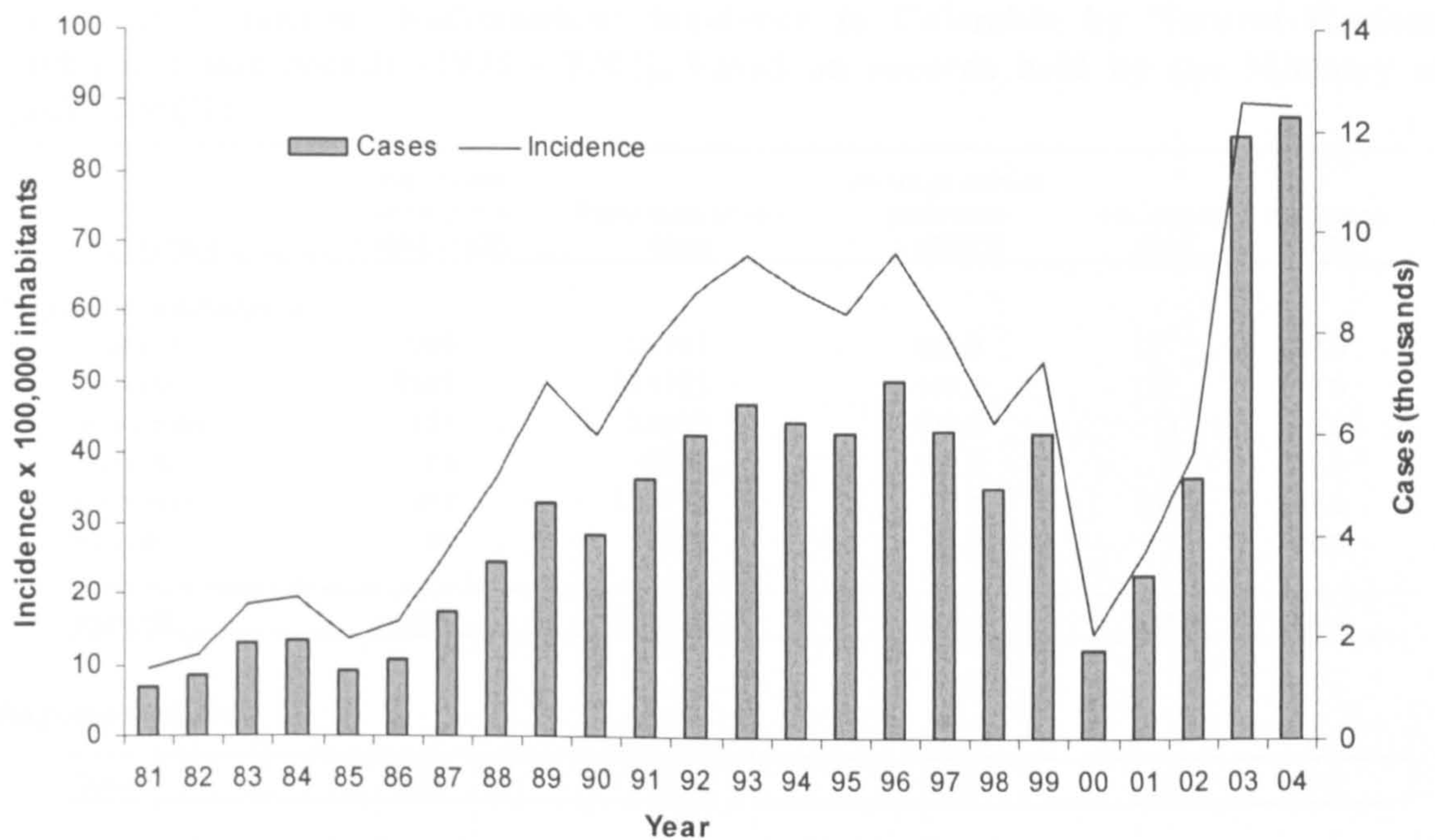


Figure 1.3 Annual incidence of cutaneous leishmaniasis in Colombia during the period 1981 - 2004, based on the reports from Programa de Enfermedades Transmitidas por Vectores, Ministry of Health (MOH).

hunting and lumbering (Weigle *et al.*, 1993). In contrast, in the sub-Andean mountainous area of Antioquia department, epidemiological surveys failed to show any difference in infection rate between men and women, or with age (Velez *et al.*, 1997). In these cases the suspected vectors could be endophagic.

Prior to this project, no analysis of risk factors had been carried out in Huila department.

Huila Department is located in the south of Colombia, including an area of 19,890 km², representing 1.74% of the country. The population of Huila is ca. 768,113 inhabitants (39.5% in rural area) (Census DANE 1993), concentrated mostly (99.3%) below 2000 m a.s.l. . The department is formed by the Colombian massif located where the Cordilleras Central and Oriental fork, the internal slopes of these cordilleras and the upper valley of the Magdalena River (Figure 1.4). The Cordillera Central has the highest altitude, up to 5600 m a.s.l. . This range is formed by volcanic material with very steep slopes. The Cordillera Oriental is lower, with an average altitude of 2500 m a.s.l. in the South and 3500 m a.s.l. in the North. It is formed by sedimentary material. The Magdalena river valley corresponds to a low plain around the river, with altitudes

Table 1.3 Cutaneous leishmaniasis incidence in Colombia by Natural Regions during the last decade (1993 - 2002), based on records held by the Ministry of Health, MOH.

Department	Total cases per 10 years (1993-2002)	Population at risk (rural)	Average annual incidence x 100000	No. cases 2002	Incidence 2002
Región de la Amazonía					
Guaviare	889	20741	428.6	157	757.0
Caquetá	2593	154781	167.5	181	116.9
Amazonas	177	13632	129.8	3	22.0
Guainía	64	6204	103.2	2	32.2
Putumayo	917	125972	72.8	165	131.0
Vaupés	56	7868	71.2	7	89.0
Subtotal	4696	329198	142.6	515	156.4
Región del Pacífico					
Chocó	2057	171776	119.7	82	47.7
Región Andina					
Norte Santander	8271	282316	293.0	262	92.8
Risaralda	1354	132454	102.2	352	265.8
Santander	4819	492220	97.9	352	71.5
Antioquia	10569	1137248	92.9	1645	144.6
Caldas	2801	301929	92.8	174	57.6
Huila	1833	289485	63.3	18	6.2
Tolima	1806	427134	42.3	158	37.0
Cundinamarca	2629	751820	35.0	304	40.4
Valle	1039	466157	22.3	181	38.8
Nariño*	1495	695512	21.5	152	21.9
Boyacá	854	668445	12.8	301	45.0
Cauca	264	602198	4.4	3	0.5
Quindío	4	63848	0.6	0	0.0
Subtotal	37738	6310766	59.8	3902	61.8
Región de la Orinoquía					
Meta	1007	168398	59.8	105	62.4
Vichada	117	26060	44.9	11	42.2
Arauca	210	49102	42.8	11	22.4
Casanare	120	60625	19.8	11	18.1
subtotal	1454	304185	47.8	138	45.4
Region del Caribe					
Sucre	1435	199422	72.0	46	23.1
Bolivar	1910	411428	46.4	154	37.4
Cesar	908	224494	40.4	56	24.9
Córdoba	1320	498422	26.5	180	36.1
Magdalena	389	303919	12.8	13	4.3
Guajira	117	118078	9.9	2	1.7
Atlántico	9	101810	0.9	1	1.0
Subtotal	6088	1857573	32.8	452	24.3
Total	52033	8973498	58.0	5089	56.7

* Half of this department belong to Region del Pacifico, but most cases of CL seem to come from the Region Andina. Cases without specified department: 793 for the all ten years period.

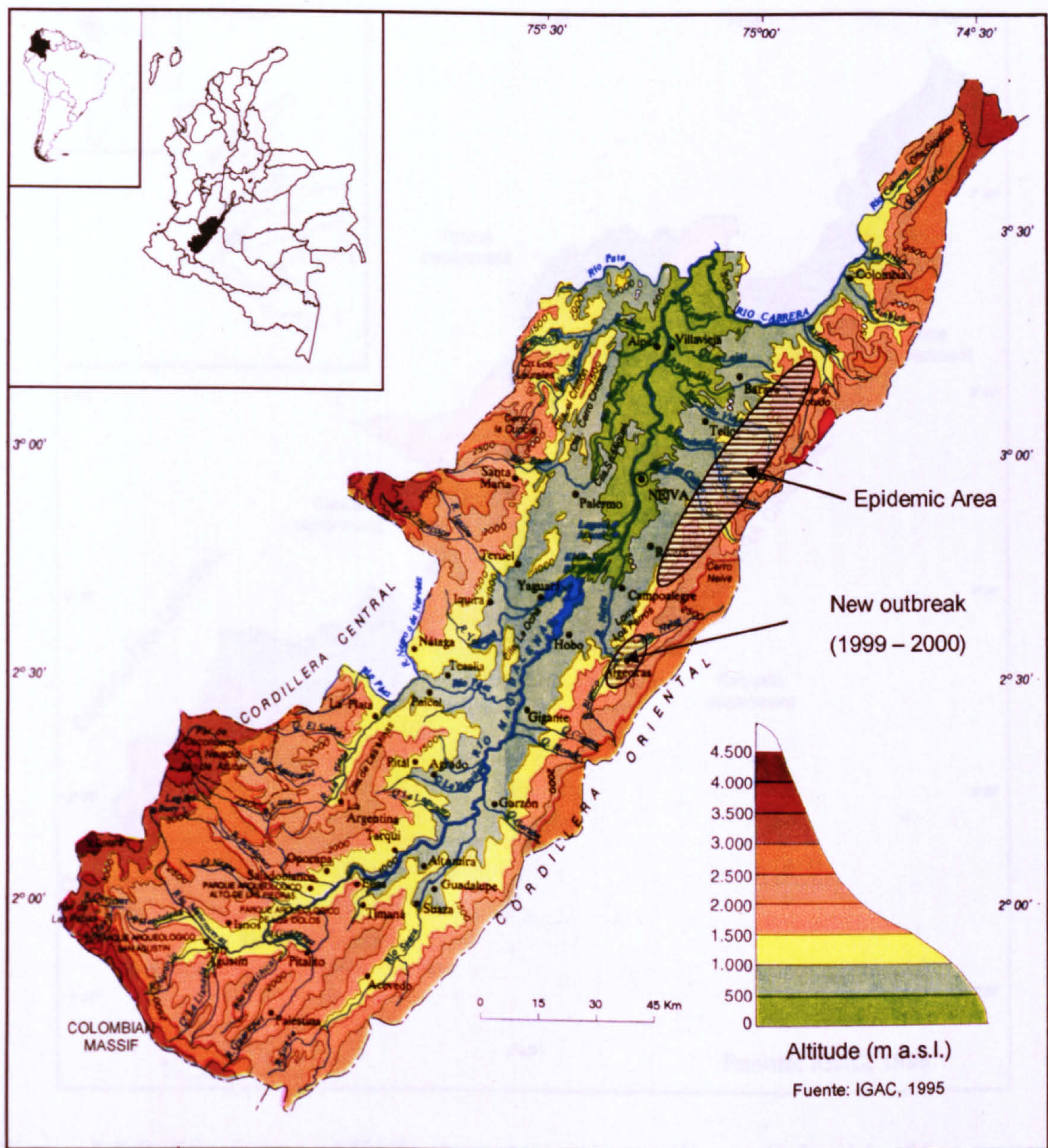


Figure 1.4 Topographic map of Huila department in southwest Colombia, including the endemic area for cutaneous leishmaniasis and the area where a recent outbreak took place. Map source: Huila, Características geográficas, IGAC, Santafe de Bogotá, 1995.

below 800 m a.s.l. (IGAC Instituto Geográfico Agustín Codazzi 1995).

Politically, Huila department is divided into 37 municipalities (Figure 1.5) all, except 2 (Villavieja and Yaguara, located on the Magdalena Valley) including at least part of the Andean mountainous area. The economy of Huila depends mainly on petroleum production (in the valley) and agriculture. The main agricultural products are rice (in the valley) and coffee plantations (in the Cordilleras area). Other crops are plantain, corn, cacao, cane, cotton and tobacco.

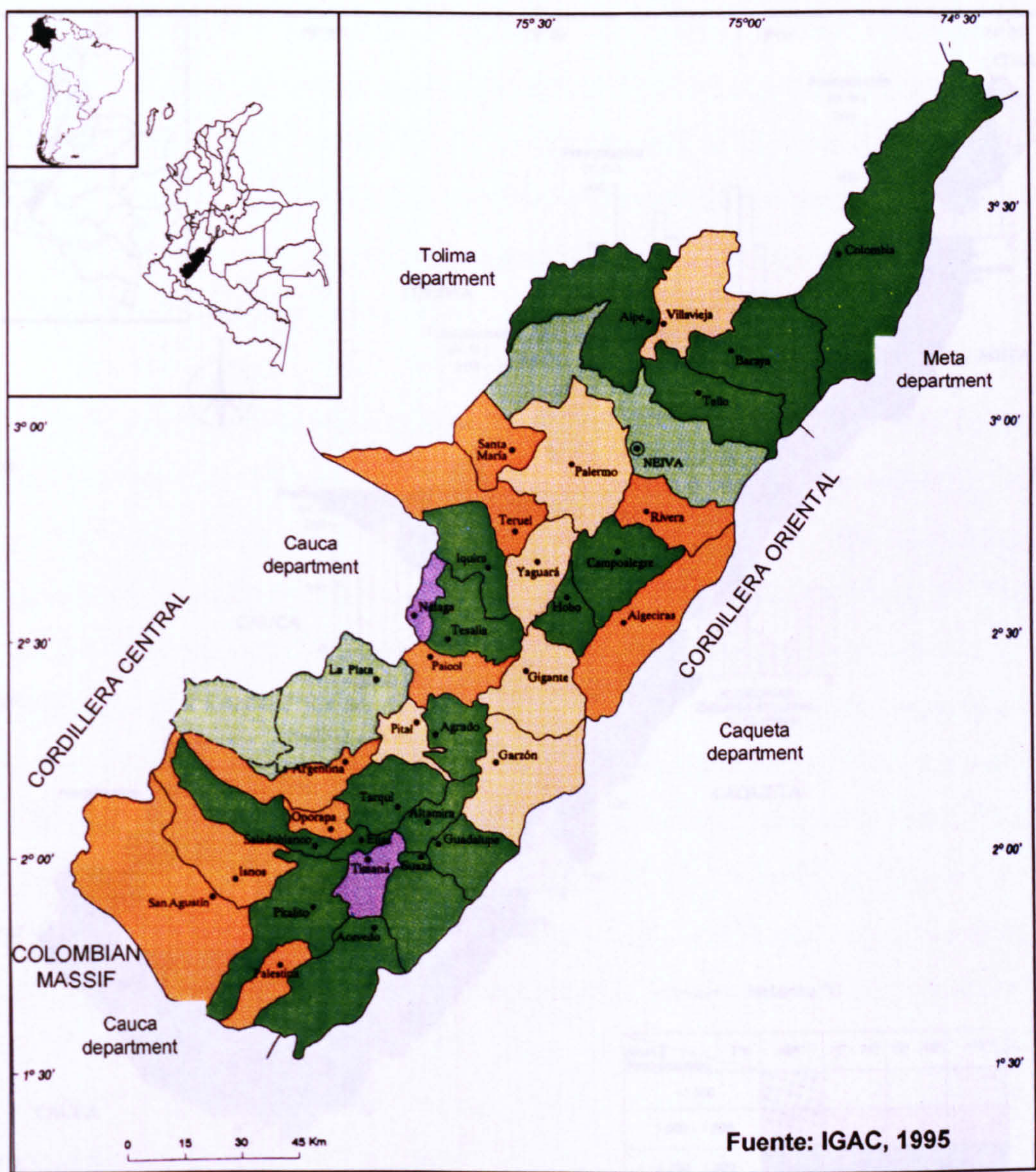


Figure 1.5 Political map of Huila department in southwest Colombia. Map source: Huila, Características geográficas, IGAC, Santafe de Bogotá, 1995.

Figure 1.6 Spatial and temporal distribution of rainfall and temperature of Huila department in southwest Colombia. Map source: Huila, Características geográficas, IGAC, Santafe de Bogotá, 1995.

The large variation in altitude in the Cordilleras, allows a wide heterogeneity in climates, from hot (temperatures > 24°C) to "Nival" (temperatures < 0°C) which are distributed vertically, as is common in the rest of the Colombian Andes. Generally the zones with less rainfall are those with the highest and the lowest altitude, while the middle part of the ranges has the highest values (1,500 – 2,500 mm). The area of lowest rainfall in the department is located in the North-East in the Cabrera river valley, where values of less than 1,000 mm are recorded (Figure 1.6). The annual rainfall has a bimodal distribution, even though there are regional variations. In general the highest rainfall is found between March and April and the lowest between January and

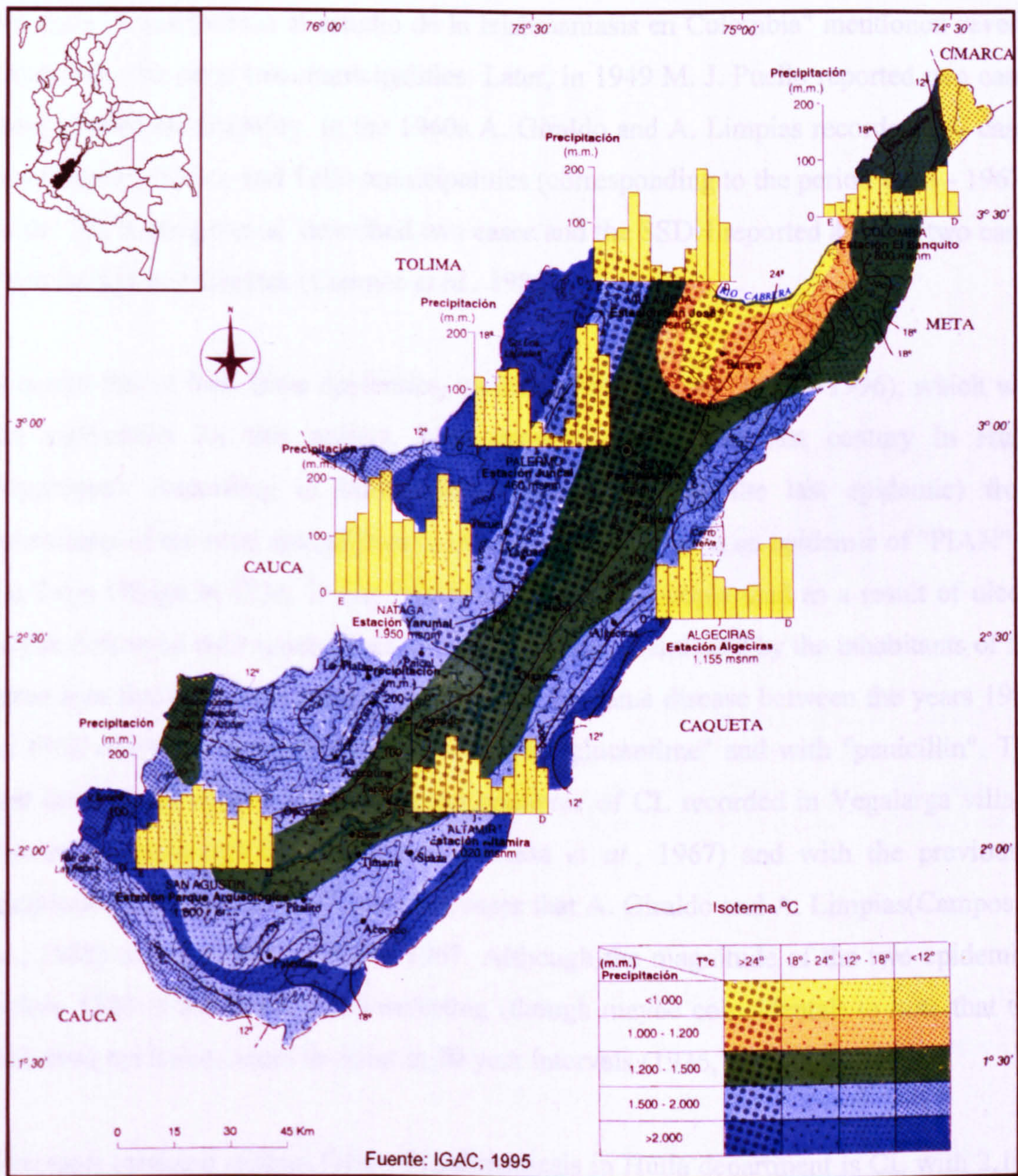


Figure 1.6 Spatial and temporal distribution of rainfall and temperature of Huila department in southwest Colombia. Map source: Huila, Características geográficas, IGAC, Santafe de Bogotá, 1995.

February, and from August to September. There are no big spatial variations in average monthly temperature, except due to altitude with an approximate decrease of 0.5 to 0.6°C for every increase in 100 m (PROCAM-INDERENA 1984). Seasonally, temperature peaks in two periods (February - March and August - September).

CL was apparently described for the first time in Huila by N. Tellez in 1890 who reported cases from Garzón and Gigante (see Figure 1.5). In 1929 J. del C. Rodriguez in

his study "Contribución al estudio de la leishmaniasis en Colombia" mentioned several cases from the same two municipalities. Later, in 1949 M. J. Puello reported two cases from Pitalito municipality. In the 1960s A. Giraldo and A. Limpías recorded 247 cases from Baraya, Neiva, and Tello municipalities (corresponding to the period 1963 - 1967). In the 70s Restrepo *et al.* described two cases and the SSDH reported another two cases from Baraya and Gigante (Campos *et al.*, 1985).

It seems that at least three epidemics, including the last one (1993 - 1996), which was the motivation for this project, have occurred during the last century in Huila department. According to information collected (during the last epidemic) from inhabitants of the rural area of Baraya municipality, there was an epidemic of "PIAN" in La Troja village in 1936. It was mentioned that three people died as a result of ulcers which destroyed their noses (Ruiz 1995). It was also mentioned by the inhabitants of the same area that there was another epidemic of the same disease between the years 1965 to 1968 where the patients were treated with "glucantime" and with "penicillin". The last information agrees with a reported epidemic of CL recorded in Vegalarga village (Neiva municipality) in 1966 (Osorno Mesa *et al.*, 1967) and with the previously mentioned high number (247 cases) of cases that A. Giraldo and A. Limpías (Campos *et al.*, 1985) recorded from 1963 to 1967. Although the magnitude of the two epidemics before 1980 is unknown, it is interesting (though maybe coincidental) to note that the last three epidemics seem to occur at 30 year intervals (1936, 1965 and 1993).

The most common clinical form of leishmaniasis in Huila department is CL with 2,108 cases (ca. 90% of all cases of leishmaniasis) reported by the Huila Health Service (SSDH) during the period 1982 to 2004 (Figure 1.7). Around 6% of the reported cases are MCL and 4% are VL. During the first years after obligatory notification was established (1982 – 1985) there was low reporting of CL cases, probably because of problems with the implementation of the programme.

During the last epidemic, which lasted for 4 years, the incidence reached a peak of 275 per 100,000 in 1994, when 426 cases were reported. Following a period of low transmission, rates gradually increased after 2000 reaching 115 cases/100,000 in 2004. Although CL has been reported in around half (46%) of the 37 municipalities in the department, their distribution is highly aggregated by municipality and even by village

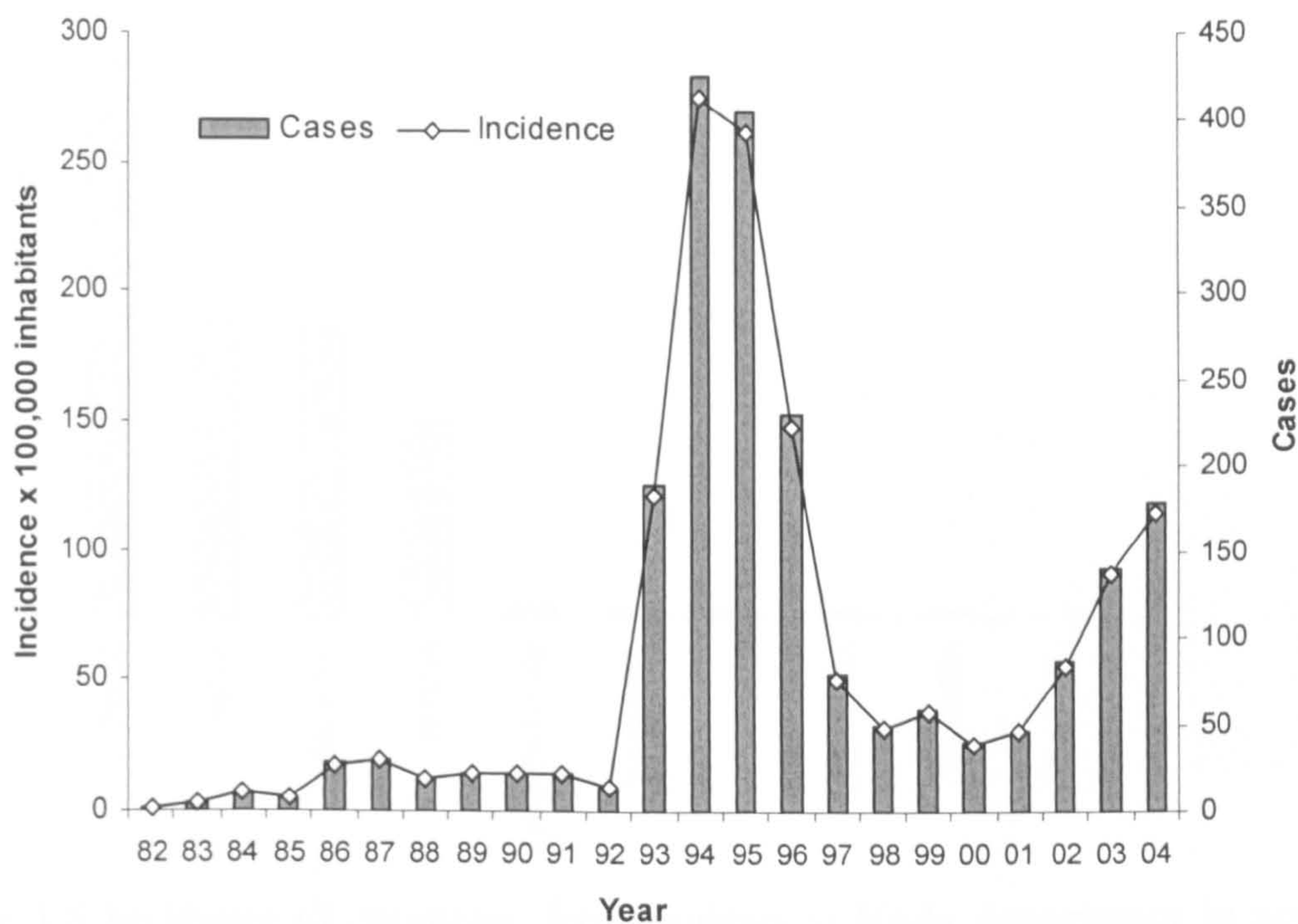


Figure 1.7 Incidence of cutaneous leishmaniasis in Huila department during the period 1982 - 2004, based on records from Secretaría de Salud Departamental del Huila, SSDH. Total cases = 2,108. Denominator for incidence was the average rural population (census 1985 and 1993) of 19 municipalities that reported cases during the study period.

(rural district) within each municipality. Based on the records 1982 - 1995, which were available at this level, the highest incidence of CL was reported in the contiguous municipalities of Baraya, Tello, Neiva and Rivera, in the sub-Andean region of the Cordillera Oriental (Figures 1.4 and 1.8). This aggregation is due mainly to the last epidemic presented from 1993 to 1996 (Figure 1.7) which was largely limited to these four municipalities.

Nevertheless, there is evidence that these municipalities actually have had foci of CL for a long time. Analysis of the SSDH records of CL cases before the epidemic (1982 - 1992) confirms that two of the municipalities, Rivera and Neiva, contributed the majority (67% and 16%, respectively) of CL cases. It is important to note that, although small in number, there was an unusual report of cases (20 cases) in Algeciras municipality from 1999 to 2000. This municipality shares boundaries with Rivera in the south of the epidemic area and had a history of only one case of CL since 1981 (Figure 1.4).

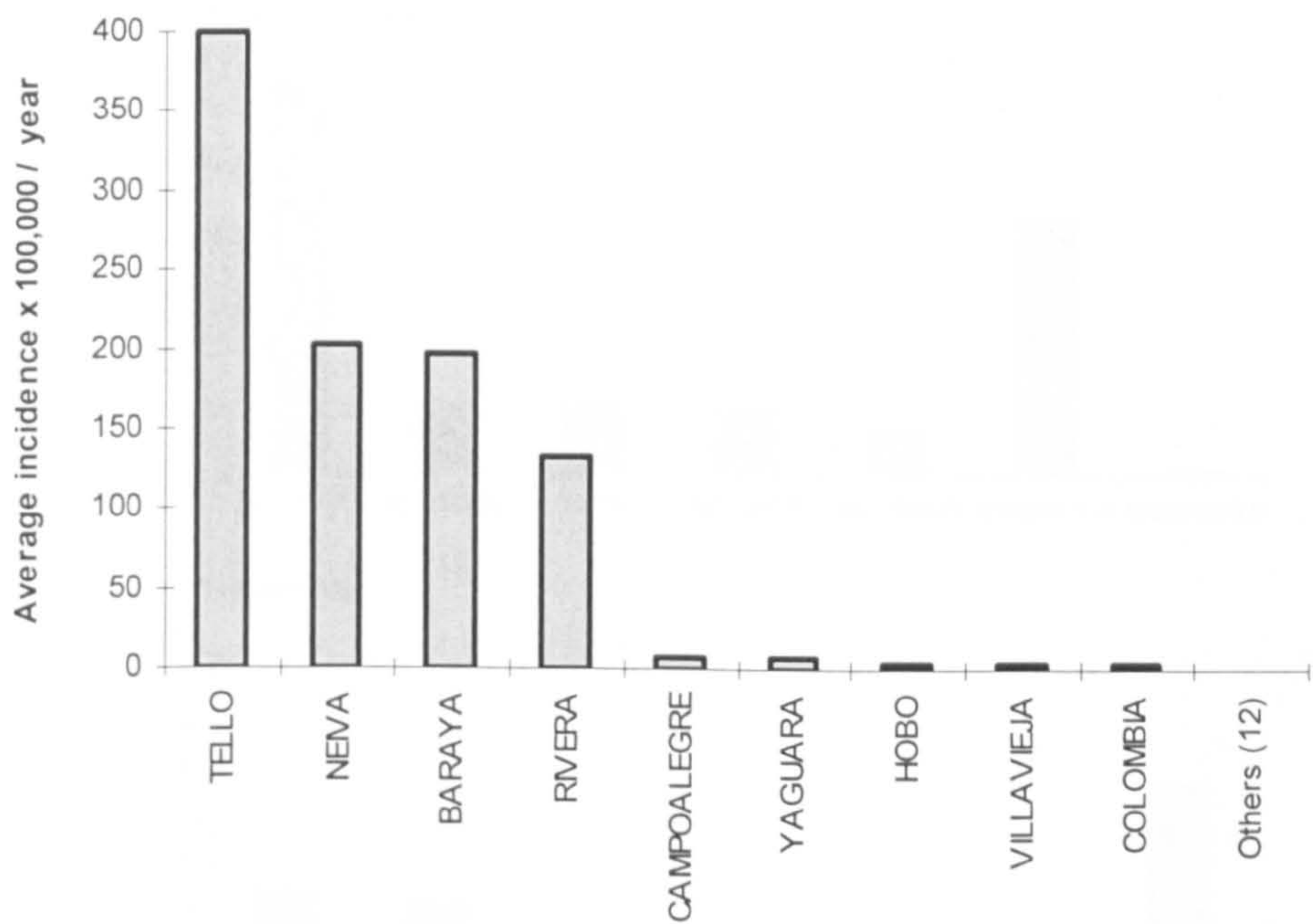


Figure 1.8 Incidence of cutaneous leishmaniasis in Huila department in positive municipalities (21/37), during the period 1982 - 1995, based on records from Secretaria de Salud Departamental del Huila, SSDH. Total cases = 1,201.

There is also notable aggregation of cases amongst villages. Less than 20% of the villages (1 to 6 villages) in each of the four epidemic municipalities contributed from 61.4% (86 of 140 reported cases in Rivera) to 70.6% (84 of 119 cases in Baraya) of the reported cases (Figure 1.9). Prior to this project there had been no attempt to address the cause of this aggregation. Previous descriptions of the foci area were limited to anecdotal observations that most cases were reported from the coffee plantations located at around 1500 m a.s.l. (Ruiz 1995; Bahamon 1995). But, this is insufficient to explain the aggregation of cases as most municipalities in Huila share this feature.

With respect to infection, little is known and there has been only one epidemiological survey carried out within the epidemic area. It detected, using the leishmanin skin test, a cumulative prevalence (based on 712 people) which ranged from 22% to 38% in six villages of two epidemic municipalities (Baraya and Tello) (S. Nicholls and C. Alvarez, personal communication).

The transmission cycle of CL in Huila department has been poorly studied. To date, two *Leishmania* species have been identified as causing CL in Huila: *Le. braziliensis* and

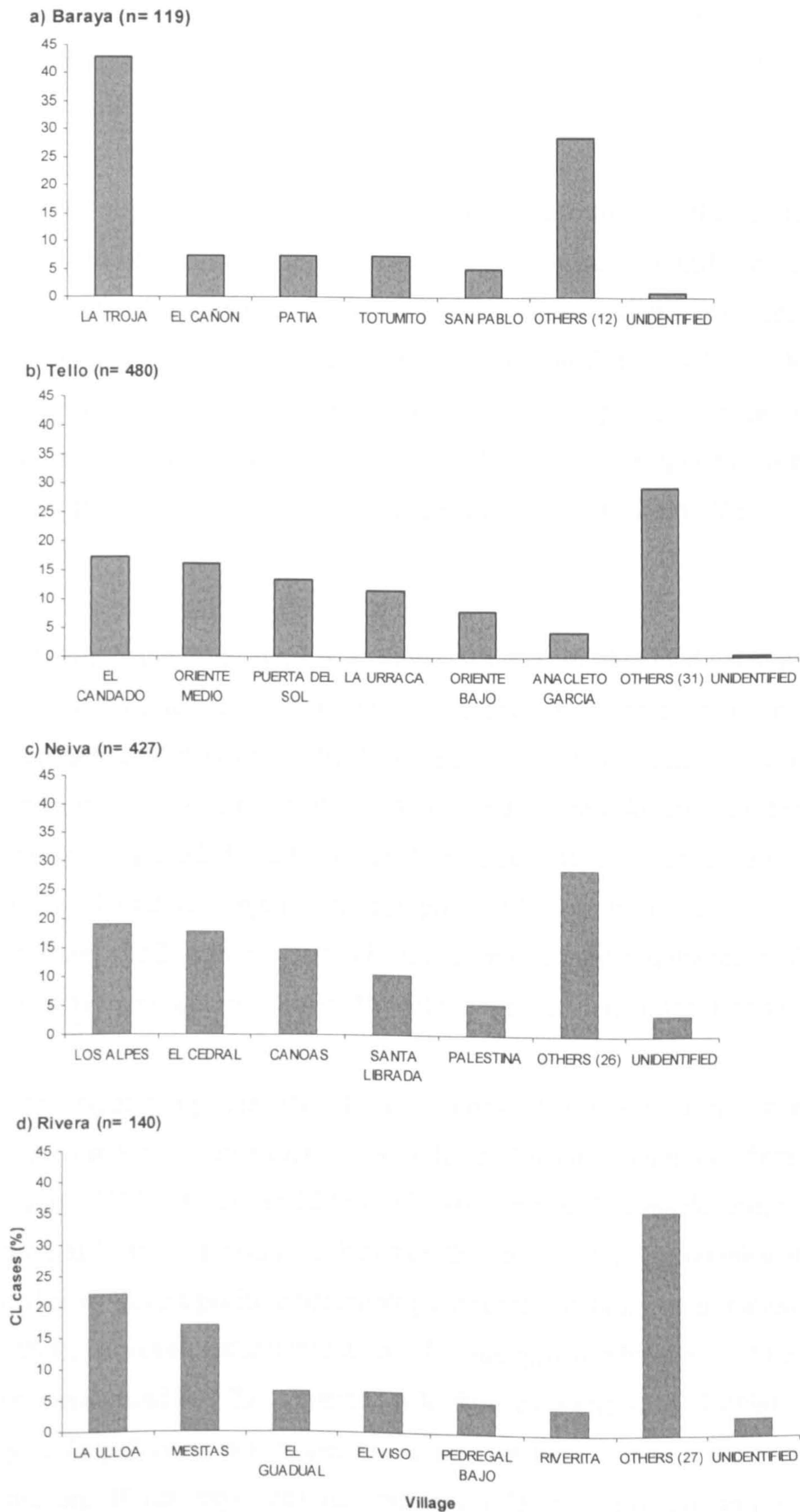


Figure 1.9 Percentage of new cases of cutaneous leishmaniasis reported in the four municipalities with the highest annual incidence of the disease, based on records from Secretaria de Salud Departamental del Huila, SSDH (1982 - 1995). n = Total number of cases per municipality.

Le. panamensis; the former within the epidemic area and the latter in the Cordillera Central, where few cases have been reported (Nicholls *et al.*, 1997; Corredor *et al.*, 1990).

Prior to 1997, the year when the Laboratorio de Entomología of the Instituto Nacional de Salud (INS) of Colombia began their studies in Huila, very little was known about CL vectors in this Department. The only significant information was the record of a new species *L. longiflocosa* Osorno-Mesa, Morales, Osorno & Hoyos 1970 (described also by studies of INS), an apparently highly anthropophilic species, collected in mountainous area of the municipalities of Tello (within the epidemic area for CL in Huila) and Rovira (in the neighbouring department of Tolima) (Young and Duncan 1994).

L. longiflocosa belongs to *verrucarum* group, which is of high medical importance. This group is formed by 40 species, half of them present in Colombia, and many of which are proven or suspected vectors of CL (Bejarano *et al.*, 2003a). The *verrucarum* group is common in mountainous areas of the Andes and Central America in degraded forest habitats (including shaded plantations such as coffee). Recent studies (prior to this PhD) by INS found that *L. longiflocosa* comprised 98% of 6,119 sandflies collected in Shannon traps, CDC traps, by human bait, and on tree trunks in the mountainous area of Baraya (La Troja village) and Tello (Roblal village) municipalities (Ferro *et al.*, 1998a).

Laboratory studies by the INS have demonstrated the vectorial competence of *L. longiflocosa* for *Le. braziliensis* as follows: Infection with *Le. braziliensis*, was achieved in 17.9% (34 / 50) to 52.9% (54 / 102) female *L. longiflocosa*, membrane fed on a promastigote suspension; a hamster that was bitten by females infected with *Le. braziliensis* gave a positive parasitological result by direct examination (Santamaría *et al.*, 1998). However, natural infections of *L. longiflocosa* have not yet been found. No parasites were found in 1,797 dissected wild females (Ferro *et al.*, 1998a). These results identify *L. longiflocosa* as a suspected vector, but further studies are required for full incrimination. If (as expected) its vectorial role is confirmed, information on its behaviour and ecology will be needed in order to design a disease control strategy. The only other species reported in Huila (prior to this PhD) which may be a suspected vector of CL is *L. nuneztovari* (Ortiz) 1954, which comprised 1.1 % of the 6,119 sandflies

collected in the study carried out in Baraya and Tello (Ferro *et al.*, 1998a). *L. nuneztovari* is the suspected vector for CL (*Le. braziliensis*) in the Yungas, Bolivia, in coffee plantations (1000 - 2000 m a.s.l.) where it is abundant and anthropophilic (Le Pont *et al.*, 1989a) and has been found naturally infected with parasites belonging to the *braziliensis* complex (Torres *et al.*, 1998). This species is also the proven vector of *Le. amazonensis* in Cajuata, also in Bolivia (Martinez *et al.*, 1999).

During the last outbreak a serological study carried out on dogs, from seven villages in Tello and four in Neiva, provided positive results for *Leishmania* in 3.3% of 800 sampled dogs (Huila Health Service 1993). However, no parasitological or molecular diagnosis of *Leishmania* species has yet been carried out on dogs, and no other wild mammals have been sampled.

The last epidemic of CL (1993 -1996) in Huila included 1,232 cases (Figure 1.7) distributed among Neiva, Baraya and Tello municipalities. The epidemic caused Huila to be classified as the department with the highest risk for CL in Colombia in 1994 (Cepeda 1997).

The cause of the last epidemic in Huila department is believed by the local health service to be due to the disease being imported by soldiers and guerrillas. These people move frequently between Huila and Caquetá departments. The latter is where the disease is thought to have originated (Bahamon 1995). A review of the CL records from SSDH (1982 - 1995 and 2000 - 2002) showed that 6.2% (119 / 1,908) of the total cases of CL recorded originated from other departments, mainly from Caquetá (56 cases), Putumayo (18 cases) and Meta (13 cases) and that, in addition to soldiers and guerrillas, peasants made an important contribution to the imported cases (28 cases) of the disease. It is important to remember that Caquetá had the second highest incidence of CL in the country (Table 1.3). According to the records of CL held by the MOH, by the time of the epidemic in Huila, the neighbouring departments of Caquetá and Putumayo also presented epidemics, 1995 to 1999 and 1995 to 1996, respectively. This hypothesis that epidemics could originate from persons infected with the disease is supported by a study that showed that sandflies can be infected by *Le. braziliensis* when they feed on active lesions of patients (Montoya-Lerma *et al.*, 1998). Other factors that could have caused

the epidemic include an increase in the population of the vector species, an increase in natural infection levels, or closer human-vector contact.

Very little is known about the risk factors of CL in Huila, and only demographic (age and sex) data are available in relation to the cases recorded by SSDH from 1982 to 1995 (Table 1.4). Overall, the sex ratio of cases was strongly male biased (males : females = 1.7 : 1), but this bias was only apparent amongst cases more than 10 years of age. Amongst children equal or less than 10 years of age the sex ratio was only 1.3 : 1. The median age for both males and females cases was 20 years, with 31.7% of females cases and 24.0% of males cases equal or less than 10 years old (suggesting significant domestic transmission). In order to calculate the relative risk in each age group, the demographic break down of the population at risk is required (but is not available). However, even without these data, the relatively high number of cases amongst males between 11 to 30 years suggests a potential high risk for this group. Of course, SSDH data may be strongly biased and unrepresentative of the relation between age or gender and infection rate. Finally, some evidence for domestic transmission came from a study of knowledge, attitude and practice (KAPs) of the population within the epidemic area. A positive association was observed between the presence of cutaneous lesions and the presence of both electricity services in the house, and the presence of hens or pigs within the peridomicile (Nicholls *et al.*, 1998). The inhabitants reported that sandfly activity occurs during the night indoors as well as in the forest and coffee crops.

It seems that there is a seasonal risk factor for CL in Huila, at least during epidemic times. This is based on a graphical analysis of the CL cases (recorded by the Hospital Local de Baraya "Tulia Duran de Borrero") from nine villages in Baraya municipality (the only municipality where detailed and organized recording of cases, as well as active surveillance for cases were carried out) which were involved in the last epidemic. The epidemic in Baraya lasted two years (1994 - 1995), including 113 cases from a population of 1,972. Figure 1.10 shows the number of CL cases grouped by month, according to the date when the clinical symptoms were first perceived by the patients, plus rainfall data from the nearest climatic station (Santo Domingo, 3° 14' N, 74° 57' W, 1300 m a.s.l.) to the main epidemic village (La Troja, 3° 11' N, 74° 57' W, 1680 m a.s.l.), during the same period of time, are also shown. All three main peaks of cases

Table 1.4 Cutaneous leishmaniasis cases in Huila department by age and gender, based on records from Secretaría de Salud Departamental del Huila, SSDH (1982 - 1995).

Age group	Female		Male		Total	
	No. cases	%	No. cases	%	No. cases	%
< 5	37	8.4	73	9.6	110	9.2
5 - 10	102	23.3	110	14.4	212	17.7
11 - 20	88	20.1	202	26.5	290	24.2
21 - 30	55	12.6	137	18.0	192	16.0
31 - 40	61	14.0	96	12.6	157	13.1
41 - 50	35	8.0	52	6.8	87	7.3
51 - 60	33	7.6	45	5.9	78	6.5
61 - 70	12	2.7	32	4.2	44	3.7
> 70	14	3.2	15	2.0	29	2.4
	438 ^a	100.0	763 ^a	100.0	1199	100.0

^a One missing data not included.

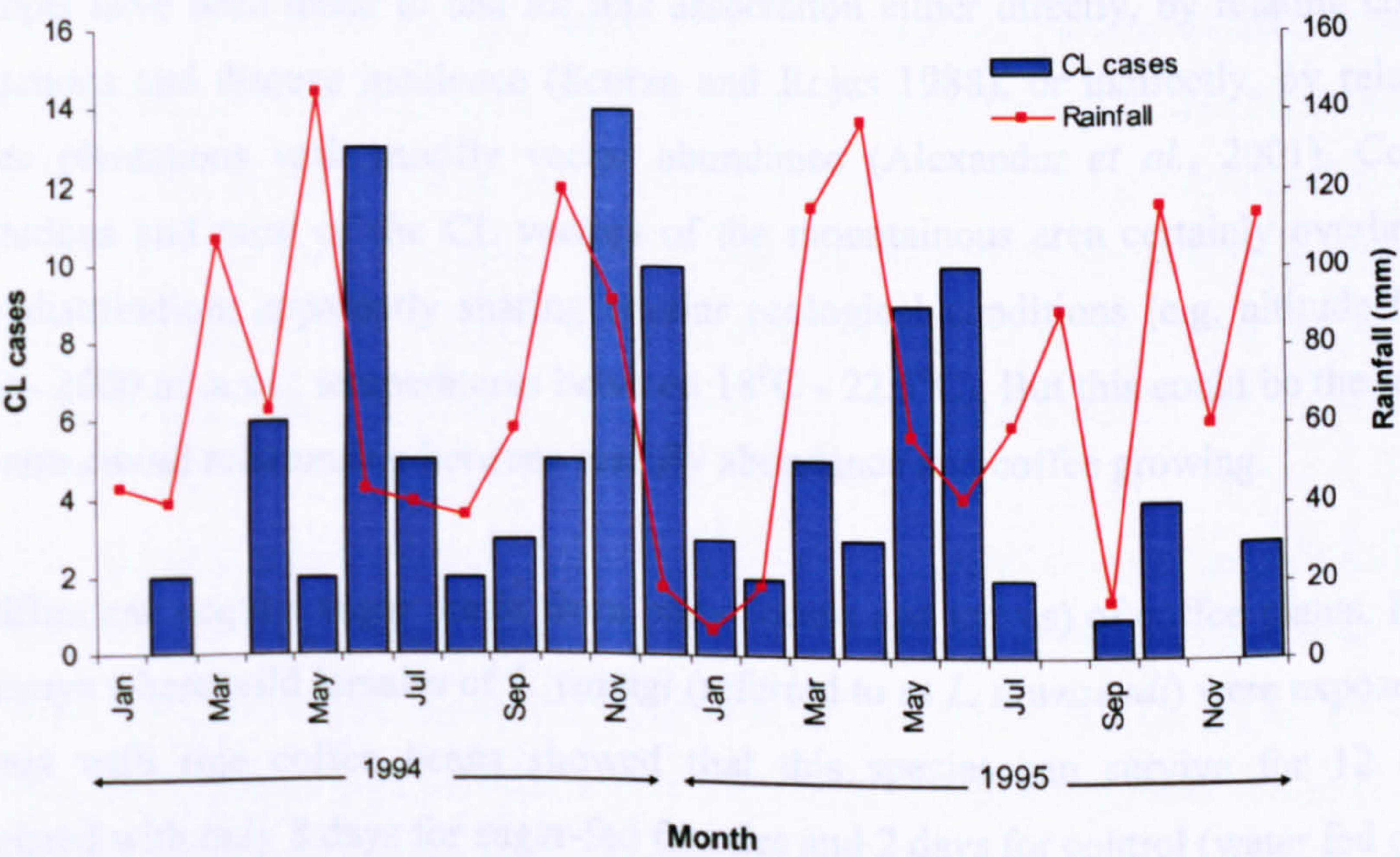


Figure 1.10 Seasonality of the epidemic peaks of CL (total cases = 113) in 9 villages (1972 inhabitants) of Baraya municipality (1994 - 1995). Cases were grouped according to the date when clinical symptoms began. Case records are from Hospital Local de Baraya "Tulia Duran de Borrero". Rainfall data are from Santo Domingo climatic station, IDEAM, Colombia.

occurred either at the beginning of the dry season or the end of the rainy season. Time series analyses found a significant correlation between monthly cases and rainfall in the previous month ($r^2 = 0.45$, $p = 0.001$). Confirmation that one month lag is the best fit for an association is shown in Figure 1.11, showing the r^2 value for lags of 0 to 9 months. Some householders are aware of an association between low rainfall and CL (Nicholls *et al.*, 1998) with 62.1% (77 / 124) of the householders that recognized CL was seasonal reporting that CL was most common in the dry seasons. Preliminary results of an ongoing study on sandfly seasonal abundance also supports the hypothesis that sandflies are most abundant during the dry seasons (A. Carvajal, personal communication).

1.3 RISK OF CL IN COFFEE PLANTATIONS

An apparent association between coffee plantations and CL in Colombia (as was explained before) has been reported, as in other areas of Latin-America, including Mexico (Sanchez-Tejada *et al.*, 2001), Venezuela (Scorza and Rojas 1988; Feliciangeli *et al.*, 1992); Ecuador (Le Pont *et al.*, 1994; Mouchet *et al.*, 1994), Bolivia (Le Pont *et al.*, 1989b; Le Pont *et al.*, 1989a); and Brazil (Alexander *et al.*, 2002). However, few attempts have been made to test for this association either directly, by relating coffee plantations and disease incidence (Scorza and Rojas 1988), or indirectly, by relating coffee plantations with sandfly vector abundance (Alexander *et al.*, 2001). Coffee plantations and most of the CL vectors of the mountainous area certainly overlap in their distribution, apparently sharing similar ecological conditions (e.g. altitude from 1000 - 2000 m .a.s.l.; temperatures between 18°C - 22.5°C). But this could be the result of a non-causal relationship between sandfly abundance and coffee growing.

Sandflies can acquire sugar meals from parts (beans and leaves) of coffee plants. Field bioassays where wild females of *L. youngi* (referred to as *L. townsendi*) were exposed to contact with ripe coffee beans showed that this species can survive for 12 days compared with only 8 days for sugar-fed females and 2 days for control (water fed only) (Scorza *et al.*, 1985). Bioassays in the laboratory with wild sugar-deprived *L. youngi*, which were exposed to coffee plants, amongst others, showed that females took sugar meals from coffee presumably by perforating the leaves (Alexander and Usma 1994).

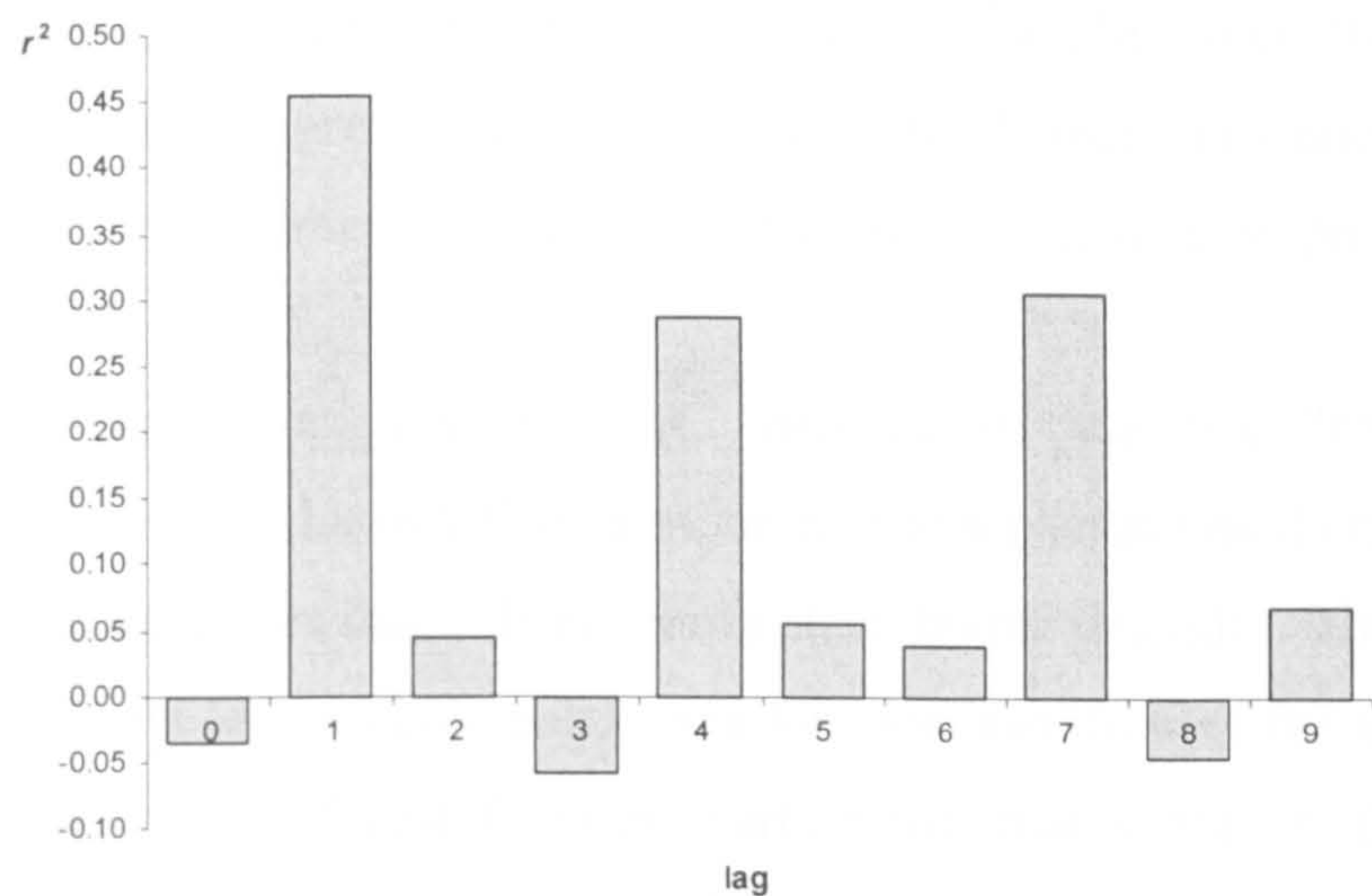


Figure 1.11 Cross-correlation between monthly rainfall and CL cases in Baraya Municipality. Lags from 0 to 9 months. Note that lag 1 gives the highest r^2 .

A causal association between coffee plantations and CL could occur if either: (1) coffee plantations favoured the presence and abundance of some of the components of the transmission cycle, such as sandfly vectors, reservoirs or parasites; and/or (2) some activities in coffee cultivation (e.g. harvesting and clearing) will increase human-vector contact.

According to the system of cultivation, coffee plantations can be classified from traditional (botanically and structurally diverse) to the least diverse and most intensive and modern plantations (Moguel and Toledo 1999). To assess the suitability of coffee plantations for harbouring sandfly vectors and favouring CL transmission, due to limitations of information, this thesis will consider only the two types of coffee plantations, representing the extreme microenvironmental conditions:

a) Traditional plantations (mainly *Coffeea arabica* of varieties "borbon" and "tipica", in Colombia), which are relatively similar to a secondary forest where the shrub layer and small trees are cleared and replaced by coffee plants and where most big trees are kept or replaced by fruit or legume trees to provide shade for the plantations. This type of plantation "preserves" the basic structure of the sub-Andean forest (mainly secondary or highly degraded primary forest), offering a large variety of microhabitats for harbouring sandflies, and a large variety of plants and animals. In this forested habitat, sandflies can find all the conditions for survival (Alexander *et al.*, 1992), including: adequate soil (rich in organic materials, with relatively stable conditions of temperature and humidity)

for breeding, resting and mating sites for adults (litter, tree trunks, buttress roots, and mammal burrows), and a variety of small mammals and humans (whose houses are generally close to or within the plantation) which could provide enough food.

b) Intensive plantations (*C. arabica* of varieties "caturra" and "Colombia", in Colombia), known also as modern or sun plantations (Perfecto *et al.*, 1996), where the coffee grows under direct sunlight, in higher densities. This plantation would appear to present less suitable conditions for most sandflies of the sub-Andean forest because of the loss of forest features, particularly tree strata. In this habitat the alteration of microclimatic conditions (including extreme variations in temperature and humidity of the air and soil); the change in the soil (drier and less rich in organic materials) and litter (formed exclusively by coffee debris) features; the reduction in mammal fauna and a direct exposure to wind, amongst other, are notable.

Hence, traditional coffee plantations may present the best conditions for sandfly population and for CL transmission simply because this plantation is a "type of secondary forest" of the sub-Andean region, which is apparently the main habitat for sandfly vectors. To demonstrate that traditional coffee is a particular "attractive" habitat for sandflies vectors, it is necessary to compare sandflies from traditional coffee plantations with neighbouring forest habitats. The only previous study which made this comparison (Warburg *et al.*, 1991) compared the sandfly fauna of two (presumably traditional) coffee plantations in leishmaniasis localities located at 1150 m a.s.l. and 1450 m a.s.l. with bushes and forested areas, respectively, located at similar altitudes. The results showed that the higher altitude coffee plantation presented a higher abundance of sandfly vectors compared with the forest, but at lower altitudes there was no apparent difference in either sandfly abundance or species composition between the coffee plantation and the bushes.

Evidence that sandfly abundance in traditional coffee plantations is significantly higher than in intensive plantations was given in a recent study where the two types of coffee plantations were compared, by simultaneous sampling, in two regions of Colombia (Alexander *et al.*, 2001). In addition, a comparison of habitats, based on species composition by UPGMA analysis, showed that sandfly fauna of the intensive

plantations were more similar to the fauna of traditional coffee plantations in the same area than to the fauna of other intensive plantations. This suggests that the population of sandflies collected in intensive plantations were temporary visitors from the neighbouring traditional coffee plantations.

In 2000 Colombia was the world's third biggest coffee producer. During that year Colombia produced 701,263 tons of coffee which corresponded to an area of 1,083,429 ha (DANE-Proyecto SISAC 2000). Coffee provides 37% of Colombian's agricultural employment, involving 3.3 million people on 566,000 farms in 590 municipalities (Anon 1999). Ninety one percent of the coffee plantations are located within the Natural Region called Region Andina. Most coffee plantations (80.2% by area) are located from 1000 to 1800 m a.s.l. (IGAC Instituto Geográfico Agustín Codazzi 1998). Intensive (as opposed to traditional) plantations cover 60% of the coffee area and contributed 80% of the total coffee production.

To explore the possible association between coffee plantations and CL, univariate comparisons of area (ha) or production (ton) of the two types of coffee plantation versus annual average incidence of CL were carried out. Comparison was by Natural Regions (to take into account the different cycles of transmission which may have occurred) and at departmental level. Coffee data corresponded to the period 1987 - 1992 (IGAC Instituto Geográfico Agustín Codazzi 1998) and epidemiological data to the period 1990 - 1992 from the MOH, using as denominator the total rural population. It was not possible to obtain data for the same period of time for both variables.

Figure 1.12 shows the comparison of coffee area and CL incidence in the five Natural Regions of Colombia. It seems clear that the Region Andina, where most of coffee area (1,091,158 ha, 94.1%), is located, has one of the lower incidences (53.8 per 100,000), while the highest incidences correspond to the Region del Pacífico (236.7 per 100,000) followed by Region de la Amazonia (88.6 per 100,000), where the area of coffee plantations is tiny (500 ha, 0.04%; and 5,642 ha, 0.5%, respectively). A similar situation applies to coffee production. Therefore, at regional level, there is no evidence of a positive association between coffee area or production and annual average incidence of CL.

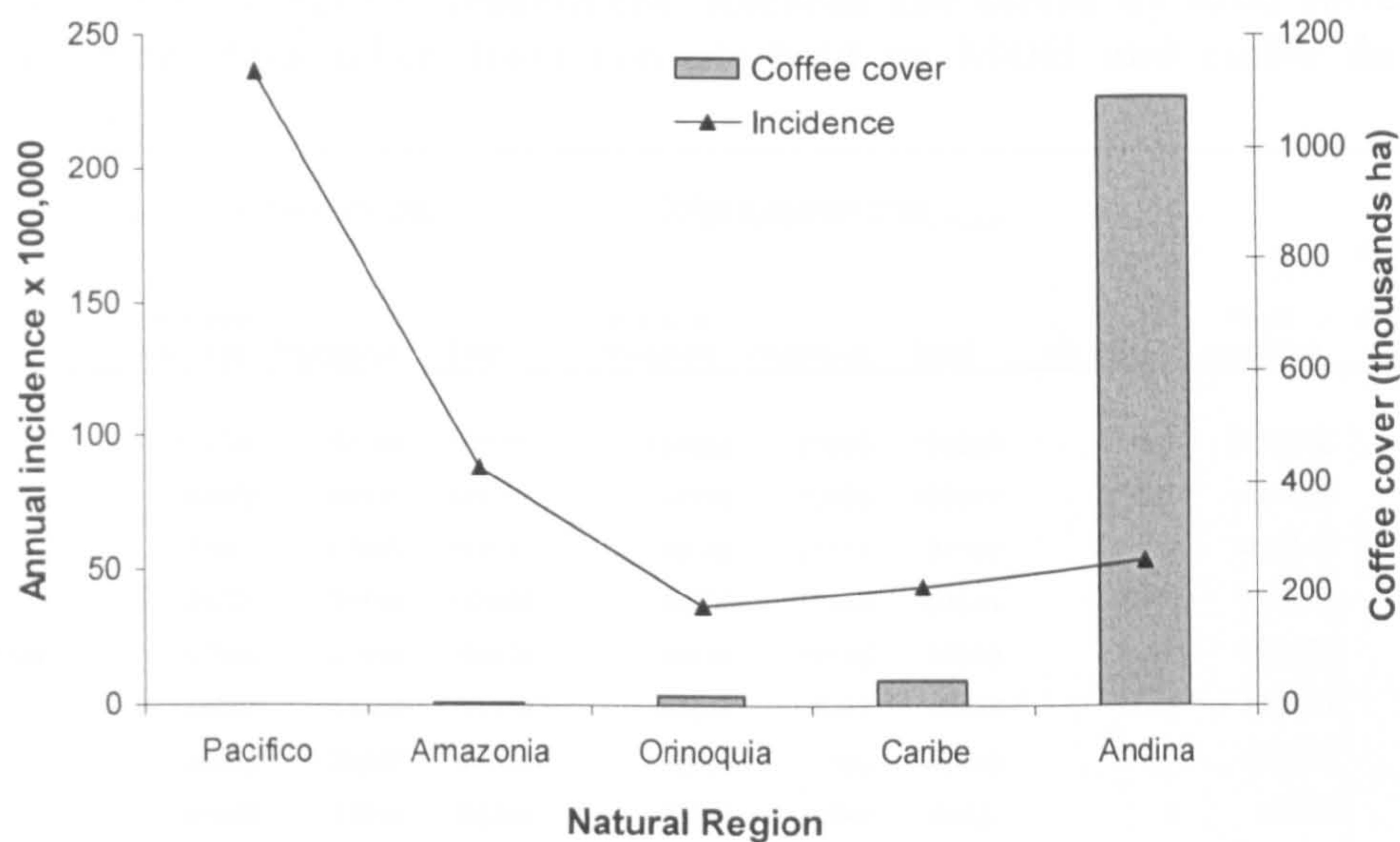


Figure 1.12 Annual average incidence of cutaneous leishmaniasis (records from MOH, 1990 - 1992) and land (ha) covered by coffee in Colombia, 1987 - 1992 (IGAC Instituto Geográfico Agustín Codazzi 1998), by Natural Regions.

By department it seems that also there is no association between coffee area and CL incidence (Table 1.5). Amongst the departments with high total area of coffee there were only two with relative high annual average incidence: Risaralda (incidence: 100.4 per 100,000) and Norte de Santander (124.2 / 100,000); but most of the other coffee growing departments have lower incidence: Antioquia (34.3 / 100,000), Tolima (13.8 / 100,000), Valle (14.0 / 100,000), Cundinamarca (7.2 / 100,000). An extreme example is Quindío department which in spite of having a high area of coffee plantations seems free of CL and apparently has a poor sandfly fauna (Alexander *et al.*, 2001). Analysis of weight of coffee produced and CL incidence gave a similar result. Spearman's correlation failed to find associations between incidence of CL and total coffee, traditional plantations and intensive plantations by area or production in the 24 departments which have coffee plantations. This does not mean that coffee is not a risk factor as: (1) the relative crude analyses were univariate and failed to account for other important variables; (2) the different transmission cycles across Colombia may vary in the relative importance of the role of coffee; and (3) the denominator (total rural population) to calculate CL incidence was underestimated in some departments because it is known that not all the rural population in a department could be at risk. A more accurate denominator is the rural population of the municipalities at risk (e.g.

Table 1.5 Coffee cover and production (1987 - 1992) and cutaneous leishmaniasis incidence (1990 - 1992) by department. Records are sorted by total coffee cover. Epidemiological data taken from records hold by MOH and coffee data from IGAC, 1998.

Department	Coffee cover (ha)			Coffee production (ton)			CL cases	Rural population	Annual average incidence of CL x 100,000 (1990-1992)
	Intensive plantation	Traditional	Total	Intensive plantation	Traditional	Total			
Antioquia	124785	52266	177051	107564	22683	130247	2980	1137248	34.3
Tolima	84352	64821	149173	78278	25669	103947	621	427134	13.8
Valle	71891	47449	119341	63048	21114	84162	305	466157	14.0
Caldas	39721	28164	117886	90707	13885	104591	1654	301929	48.0
Cundinamarca	57594	37995	95550	49415	16185	65600	431	751820	7.2
Risaralda	59653	15902	75556	63351	6567	69918	798	132454	100.4
Cauca	39450	35397	74848	32663	103	42963	117	602198	2.5
Quindío	49249	19744	68994	50874	11747	62621	0	63848	0.0
Huila	41533	25326	66859	31523	5647	37170	47	289485	1.3
Santander	36556	22746	59303	34216	7870	42086	548	492220	23.8
Norte Santander	21881	26982	48863	18599	7527	26126	1613	282316	124.2
Cesar	5776	15751	21527	3997	4568	8565	293	224494	13.4
Nariño	15860	3045	18905	19037	870	19907	989	695512	11.4
Boyacá	9603	9226	18829	7000	2390	9390	90	668445	1.2
Meta	2848	13905	16754	1651	5047	6698	271	168398	30.1
Magdalena	4089	12267	16357	3100	3251	6351	170	303919	5.8
Caquetá	855	4487	5342	550	1346	1896	435	154781	32.5
Guajira	1676	3221	4897	1456	1008	2464	90	118078	13.0
Casanare	102	2443	2545	64	408	472	6	60625	2.7
Chocó	273	227	500	209	46	255	1220	171776	77.6
Bolívar	5	445	450	2	60	62	515	411428	12.7
Putumayo	120	180	300	79	49	128	126	125972	11.6
Arauca	9	141	150	4	23	27	28	49102	10.9
Córdoba	5	15	20	4	2	6	425	498422	9.6
Amazonas	0	0	0	0	0	0	74	13632	19.6
Guainía	0	0	0	0	0	0	18	6204	26.9
Guaviare	0	0	0	0	0	0	200	20741	157.5
Vaupés	0	0	0	0	0	0	22	7868	42.4
Atlántico	0	0	0	0	0	0	0	101810	0.0
Sucre	0	0	0	0	0	0	930	199422	54.3
Vichada	0	0	0	0	0	0	25	26060	7.7

municipalities that report at least one CL case each five years) in a department. Unfortunately, this information was not available.

There are only two published attempts to relate quantitatively coffee plantations with CL. In Venezuela, Scorza and Rojas (1988) compared the number of cases of CL during the period 1975 - 1978 with coffee production and area covered by coffee

plantations during the same period by state. The results showed that there was a highly significant correlation between both coffee area ($r = 0.69$, $p < 0.01$) and production ($r = 0.78$, $p < 0.001$) with CL cases. However, the study failed to adjust for variation in the population at risk. In a cross-sectional study to detect CL risk factors in Llaucano inter-Andean valley, Perú, Zorrilla *et al.* (2005) found that the presence of coffee plantations (unspecified type) increased the risk of CL by 7.8 (C.I. 3.6 – 17.2). Although the analysis was only univariate, the results indicate that the role of coffee can be demonstrated at local level where there is less heterogeneity to confound the analysis.

One of the aims of this project was to investigate the role of coffee growing as a risk factor for sandfly vectors at a relatively high resolution, i.e. within a single department; Huila.

In 1996, Huila was the tenth most important (of 24) department for coffee production with 37,170 tons of coffee (4.5% of the national production), in an area of 71,523 ha (Federación Nacional de Cafeteros de Colombia 1997). Intensive coffee (as opposed to traditional) corresponds to 78% of area which contributes 84.8% of the departmental production. Coffee is grown in the Andean area of Huila, which includes three (Cordillera Oriental, Cordillera Central and Colombian Massif) of the four morphological regions in which Huila is divided, including 35 of the 37 municipalities in the department. No coffee is grown in the Magdalena valley region (Table 1.5). The region with more area of coffee plantations is the Cordillera Oriental region where 50.4% of all coffee is grown. By altitude, 98.5% of the coffee area is located from 1000 to 2000 m a.s.l. . Municipalities with the largest area of coffee are Pitalito and Garzón, both accounting for 20.1% of the coffee area of Huila and they are also two of the biggest producers joined by Gigante.

The possible association between coffee plantations and CL was carried, in this case, at municipality level by comparison of area (ha) with coffee and by each of the two types, recorded as varieties "tipica", "Colombia" and "caturra" (most of "tipica" is grown in traditional plantations and most of "Colombia" and "caturra" grown in intensive plantations), with the annual average incidence of CL. Coffee data were taken from the Encuesta Nacional Cafetera 1993 - 1996 (Federación Nacional de Cafeteros de Colombia 1997), and epidemiological data were obtained from the records 1990 - 1995

hold by SSDH, using as denominator the rural population. Statistical analysis was the same as used in the comparison at departmental level.

As can be observed in Table 1.6, the relationship between coffee plantations and CL incidence in Huila can not fully explain the limited distribution of the epidemic area, since coffee producing areas within the department spread along the whole mountainous area including 35 of the 37 municipalities. Even though most of the coffee production area (36,063 ha) is located on the Cordillera Oriental, which presents the highest incidence (328.3 per 100,000), it is not concentrated in the municipalities (Baraya, Tello, Neiva and Rivera) comprising the epidemic area (which account only for 9.9% of the coffee area). Furthermore, the biggest coffee areas in this cordillera are located in municipalities where none (Garzón, Acevedo, Algeciras, Timana) or very few (Gigante) cases of CL were recorded during the study period (Table 1.6). By coffee type, a similar situation is observed. Spearman's correlation, using data from the 35 coffee producing municipalities, confirmed the apparent lack of association between CL incidence in the whole study period and coffee growing (total, traditional or intensive). The same results were obtained when CL incidence before (1990 - 1992) or during the epidemic (1993 - 1995) was tested.

In conclusion, no evidence was found for a correlation between CL incidence and coffee growing at National level or within Huila department. On the other hand, CL incidence must therefore be strongly influenced by other factors, such as climate, soil types and the particular features of primary and secondary vegetation, which might determine the vector population size, its age structure, presence of reservoirs, and hence transmission rates of CL. But it remains possible that more refined studies, such as risk factor studies would be successful in finding a possible positive association with coffee plantations.

1.4 CUTANEOUS LEISHMANIASIS CONTROL STRATEGIES IN THE ANDEAN REGION, COLOMBIA AND HUILA DEPARTMENT

The use of insecticides has played the major role in CL control world wide, especially residual insecticide spraying of houses with organochlorines, organophosphates, carbamates and currently pyrethroids (Oliveira and Melo 1994). House spraying has been particularly effective for controlling peridomestic and endophilic

Table 1.6 Coffee cover and cutaneous leishmaniasis (CL) incidence by municipality and region in Huila department. Data taken from: Encuesta Nacional Cafetera, 1993 -1996; census 1993, DANE; and epidemiological records held by Secretaria de Salud Departamental del Huila, SSDH (1990 -1995).

Municipality	Coffee cover by variety (ha)			CL cases (1990 -1995)	Rural population (1993)	Annual average incidence of CL x 100000 (1990 - 1995)
	"tipica"	"colombia" and "caturra"	total			
Cordillera Central						
Paicol	129	540	668	1	3212	5.2
Teruel	482 ^a	1280 ^a	1762 ^b	1	3308	5.0
Pital	394	1742	2136	2	7384	4.5
Palermo	806	1026	1832	2	9799	3.4
Santa María	872	1244	2117	1	6406	2.6
Agrado	160	432	592	0	3930	0
Aipe	449	456	904	0	4331	0
Iquira	473 ^a	1259 ^a	1732 ^b	0	4135	0
La Argentina	466	1069	1535	0	5996	0
La Plata	876	3819	4696	0	20014	0
Nataga	141 ^a	374 ^a	515 ^b	0	3575	0
Tarqui	399	1771	2170	0	9294	0
Tesalia	68 ^a	180 ^a	248 ^b	0	4093	0
Subtotal	5714	15192	20906	7	30109 ^c	3.9
Macizo Colombiano						
Saladoblanco	454	1399	1853	1	6720	2.5
San Agustin	716	2121	2837	2	15454	2.2
Pitalito	806	7378	8184	2	26561	1.3
Elias	127	421	548	0	1612	0
Isnos	265	1179	1444	0	14558	0
Oporapa	263	1265	1527	0	5651	0
Palestina	530	1886	2416	0	5743	0
Subtotal	3162	15649	18811	5	48735 ^c	1.7
Cordillera Oriental						
Tello	1155	1703	2858	475	9828	805.5
Neiva	1184	1704	2888	403	13599	493.9
Baraya	433	454	887	118	4291	458.3
Rivera	200	399	599	61	8526	119.2
Campoalegre	218	703	921	6	6888	14.5
Gigante	332	3327	3659	2	10931	3.0
Acevedo	339	5263	5602	0	15090	0
Algeciras	664	2974	3638	0	11016	0
Altamira	59	76	134	0	727	0
Colombia	939	414	1352	0	7394	0
Garzón	1123	5047	6170	0	18383	0
Guadalupe	361	1427	1787	0	7864	0
Hobo	317	387	703	0	1435	0
Suaza	493	1207	1701	0	6218	0
Timana	222	2941	3163	0	13817	0
Subtotal	8038	28025	36063	1065	54063 ^c	328.3
Magdalena Valley						
Yaguara	0	0	0	1	911	18.3
Villavieja	0	0	0	0	4606	0
Subtotal	0	0	0	1	911 ^c	18.3
Total	15751	55773	71523	1078	133818	134.3

^a Data were not available. Numbers were calculated based on the percentage of each coffee type for the region; ^b Data taken from Comité Departamental de Cafeteros del Huila (1995 - 1996); ^c Sum included only municipalities that reported cases (i.e. rural population at risk); ha: hectares.

vectors (Davies *et al.*, 1994; Le Pont *et al.*, 1989c; Kelly *et al.*, 1997). However, the main inconvenience for CL control, as for malaria, is the problem of sustainability. There remains a need to search for alternative control measures which could be maintained in the long run (Davies *et al.*, 2000b).

The use of insecticide-treated bednets for malaria vectors has become widespread across the world and its efficacy has been widely proved in Africa where significant reductions in child mortality and disease incidence have been achieved (Lengeler *et al.*, 1996). The advantage of insecticide-treated bednets for sustainability as compared with spraying are: a) the cost of the intervention is lower (Curtis *et al.*, 1998), specifically there is a notable reduction in insecticide cost, b) the community could have a substantial participation in the control avoiding dependence on a control program, c) no specialized equipment and personnel are needed. On the other hand, it should be noted that the participation of the community requires, at least during the first years, the introduction of programmes of motivation and education in order to guarantee that bednets are used all the time during the periods of risk identified and that they are kept in optimal conditions of use, including insecticidal effectiveness. All this requires an additional cost which should be considered.

Insecticide-treated bednets may well be a practical control measure for leishmaniasis vectors. Trials in Afghanistan (Reyburn *et al.*, 2000) and Syria (Tayeh *et al.*, 1997) indicate a significant reduction in incidence of anthroponotic CL. In Colombia, insecticide-treated bednets were shown, in a small experimental trial, to reduce the indoor biting rate of sandfly vectors of CL in the coffee plantation areas of Valle del Cauca department (Alexander *et al.*, 1995c). Treated bednets are currently provided free of charge as a vector control measure for leishmaniasis, by the health service of some departments (e.g. Antioquia, Sucre, Boyacá, Cundinamarca, Santander and Huila), though their impact has not been evaluated. The recent introduction of insecticide treated bednets for malaria control by the MOH could also provide protection for leishmaniasis in areas where the two diseases are present.

In any particular endemic area for leishmaniasis the effectiveness of insecticide treated bednets will depend on (a) the degree of anthropophily and endophagy of the vector species; (b) the temporal overlapping between the time of indoor biting and the sleeping

habits of the people living in the house; (c) the continued use (at least when a specific seasonal risk has been detected) and by all the community of the treated bednets; and (d) the degree of insecticide resistance of the sandfly species.

In Huila department, the control and prevention measures for CL are currently carried out by the municipal Health Services in coordination with the Secretaría de Salud Departamental (SSDH), and focus on the treatment of patients, vector control and educational campaigns. Vector control has been implemented by the sanitation control programme and it involves mainly large scale residual insecticide application inside and around houses (including external walls and animal shelters). However, there has also been occasional ultra low volume (ULV) insecticide spraying. Spraying records from SSDH exist only from 1993, when the last epidemic started. Spraying appears to be carried out in an irregular fashion, as is common for all the Andean areas where CL is endemic (Davies *et al.*, 2000b). The choice of areas to spray depends, mainly, upon the report of an unusual high number of CL cases from a specific village, along with a request by the communities. Frequency is usually only once. The most regularly used insecticide has been sumithion (40% fenitrothion) applied at 200 mg/m² dose indoors. However, since the beginning of 1998, ICON (lambda-cyhalothrin) has been in use. Recently (1999), the Health Service of Neiva municipality (NHS) introduced insecticide-treated bednets in nine villages, and the SSDH (2000) used the same measure to control a small outbreak of CL in one village of Algeciras municipality. In both interventions, approximately 1,400 treated bednets (deltamethrin, 25 mg/m²) were delivered. Nevertheless, due to limited resources coverage has been relatively low, and no re-impregnation campaign has been carried out.

Until now, the sanitation authorities of the region lack a clear policy as to where, when and how spraying should be done in order to achieve the best results. Indeed, it is not known if this measure, or the recently introduced insecticide-treated bednets, is effective, since no monitoring or evaluation of the impact has been undertaken. This is due, amongst other factors, to the lack of basic knowledge on transmission cycles of the disease in the epidemic area of Huila, particularly in relation to the vector (incrimination, vector biology, ecology and behaviour) which is the basis for the establishment of any control programme.

1.5 RATIONALE AND OBJECTIVES

Seventy three percent (37,738) of all CL cases recorded in Colombia during the last decade were from the Region Andina. Vector control programmes are hampered by the remarkable diversity of transmission cycles and the limited knowledge of the cycles in the majority of foci. From at least 13 main highly endemic foci of CL, mostly within the Region Andina, reported in Colombia in 1986 by the MOH, only three have been studied in detail, and only three sandfly species of the current twelve recorded sandflies species involved as vectors in the Region Andina have been confirmed as proven vectors. Hence, field studies in these remaining foci (and other important new foci, e.g. in Cundinamarca and Tolima departments) have been prioritised. Such studies, it is hoped, will improve knowledge of the risk factors for infection and so aid the design and implementation of cost-effective control measures. The identification of possible patterns in transmission could be used to extrapolate control measure recommendations to areas with similar ecological conditions within the Andes. This thesis was designed to improve the understanding of the transmission cycle of CL in the sub-Andean region of Huila department and to explore alternative control measures to house spraying for CL control. The main objectives of the thesis were:

- 1) To identify suspected sandfly vectors of CL in the sub-Andean region of Huila department.
- 2) To provide incriminatory evidence for the suspected sandfly vectors.
- 3) To describe quantitatively the spatial variation in abundance of the suspected sandfly vector and explain this variation based on their ecological determinants.
- 4) To provide evidence to clarify the role of coffee plantations as a risk habitat for CL.
- 5) To identify determinants of variation in indoor abundance of the suspected sandfly vectors.
- 6) To identify risk factors (demographic, house features, host abundance and surrounding habitats, and entomological) for CL at household level.
- 7) To evaluate insecticide-treated bednets as an alternative to house spraying for the control of CL.
- 8) To describe the knowledge, attitudes and practices that the community of the epidemic area for CL have on CL and its control, with emphasis in sandfly control.

The main findings of each chapter are summarized in annexe 49.

2 PROBABLE SANDFLY VECTORS AND THEIR ECOLOGICAL DETERMINANTS

2.1 INTRODUCTION

2.1.1 Background on sandflies in Huila department

Although CL is an important disease in Huila department, the knowledge about sandfly vectors is relatively poor. Taxonomic information on sandflies in Huila is limited, mainly due to occasional sampling in some municipalities. Before the present PhD, 14 sandfly species had been reported, seven of which are anthropophilic (Table 2.1). Six of these species have probable epidemiological importance: *L. longipalpis*, vector of visceral leishmaniasis in the Magdalena valley (Ferro *et al.*, 1995); *L. longiflocosa*, *L. gomezi*, *L. columbiana*, and *L. lichyi* are considered vectors or suspected vectors of CL in the Colombian Andes (Ferro *et al.*, 1999; Velez *et al.*, 1991; Muñoz-Mantilla 1998; Montoya-Lerma *et al.*, 1999; Alexander *et al.*, 1995d; Montoya *et al.*, 1990); and *L. nuneztovari*, vector of CL in Bolivia (Martinez *et al.*, 1999; Torres *et al.*, 1998). Incriminatory evidence for possible vectors in Huila (prior to this PhD) was also scarce, with *L. longiflocosa* proposed as a possible vector (by INS) based solely on its high abundance in two localities of the epidemic area and on its vectorial competence (Chapter 1, section 1.2). Hence, this chapter describes studies aiming to provide further incriminatory evidence for the likely vector(s) of CL in Huila, and to provide a better understanding of the ecology of the suspected vector(s).

2.1.2 Overview on ecological determinants for sandflies

Knowledge of the spatial and temporal distributional patterns of sandflies is important to identify the limits of the CL foci. These patterns of distribution are the results of the interaction of environmental variables (physical and biotic). Spatial patterns are commonly aggregated or contagious for many animal and plant populations, including sandflies (section 2.3.2 and Chapter 3, section 3.4.1), principally due to the uneven distribution of the environmental determinants. There may also be a tendency for some

Table 2.1 Sandfly species reported for Huila department prior to the present study.

Species	Municipality	Reference
Verrucarum group		
<i>Lutzomyia longiflocosa</i> Osorno, Morales, Osorno & Hoyos, 1970 ^a	Tello	Young, D., 1979
	Baraya	Santamaría et al., 1998
<i>Lutzomyia columbiana</i> (Ristorcelli & Vanty, 1941) ^a	Baraya, Tello	Ferro et al., 1998a
<i>Lutzomyia nuneztovari</i> (Ortiz, 1954) ^a	Timana	Young, D., 1979
Subgenus Lutzomyia		
<i>Lutzomyia lichyi</i> (Floch & Abonnenc, 1950) ^a	Suaza	Young, D., 1979
<i>Lutzomyia longipalpis</i> (Lutz & Neiva, 1912) ^a	Baraya	Young, D., 1979
	Rivera, Yaguara Campoalegre, Palermo, Paicol	Ferro, C. (personal communication) ^b
<i>Lutzomyia gomezi</i> (Nitzulescu, 1931) ^a	NS	Young, D., 1979
	Rivera, Palermo	Ferro, C. (personal communication)
Migonei group		
<i>Lutzomyia dubitans</i> (Sherlock, 1962)	NS	Young, D., 1979
	NS	Young & Duncan, 1994
	Tello	Ferro, C. (personal communication)
<i>Lutzomyia walkeri</i> (Newstead, 1914)	Tello ^c	Montoya-Lerma & Ferro 1999
Subgenus Psathyromyia		
<i>Lutzomyia punctigeniculata</i> (Floch & Abonnenc, 1944)	NS	Young, D., 1979
Subgenus Micropygomyia		
<i>Lutzomyia cayennensis</i> (Floch & Abonnenc, 1941)	NS	Young, D., 1979
	Yaguara, Palermo Teruel	Ferro, C. (personal communication)
<i>Lutzomyia atroclavata</i> (Knab, 1913)	NS	Young, D., 1979
	Rivera, Campoalegre Teruel	Ferro, C. (personal communication)
Subgenus Oswaldoi		
<i>Lutzomyia trinidadensis</i> (Newstead, 1922)	Baraya	Young, D., 1979
	Yaguara, Rivera Palermo, Teruel, Paicol	Ferro, C. (personal communication)
	Neiva	Osorno-Mesa et. al., 1967
Pilosa group		
<i>Lutzomyia pilosa</i> (Damasceno & Causey, 1944)	La Plata	Young, D., 1979
	Baraya, Pitalito Yaguara	Ferro, C. (personal communication)
Ungrouped species		
<i>Lutzomyia pia</i> (Fairchild & Hertig, 1961) ^a	Tello ^c	Montoya-Lerma & Ferro 1999

^a Anthrophilic or opportunistic human biter species; ^b Based on unpublished information from Laboratorio de Entomología, Instituto Nacional de Salud, Bogotá, Colombia; NS: Municipality no specified; ^c Municipality data taking from the same source given in " ^b ".

species to aggregate and thus produce a contagious distribution (Elliott 1977). Environmental determinants for sandflies are not only risk factors for sandflies but also for the diseases they transmit. Environmental determinants associated with sandflies can be classified according to spatial scale: “regional determinants” or “local determinants”. The former include climate, topography, soil and vegetation characteristics that can be remotely detected for a given sandfly sampling location by interpolation from satellite images (or meteorological stations); in contrast, “local determinants” include physiognomic structural features (tree strata, cover, buttresses and holes on the trees, and presence of climbers), litter, flora and microclimatic conditions, i.e. features which can only be recorded by direct observation of the sampling location. In Colombia, for example, the abundance of *L. evansi* has been apparently associated with the presence of *Garcia nutens*, *Ficus affinis maxima* and *Guazuma ulmifolia* (local determinants) (Prado and Travi 1998) and with semidry climates (regional determinant) (Velez 1995). In neighbouring Panama, the abundance of several sandfly species have been associated with local determinants. For instance, emergent adults of *L. pessoana*, *L. panamensis*, *L. gomezi* and *L. insolita* were associated with large trees of the genus *Anacardium*; *L. trapedoi* and *L. rorotaensis* with large lianas of the genera *Ouroparia* and *Sabicea* (Rutledge and Ellenwood 1975a); *L. trapedoi*, *L. ylephiletor* and *L. shannoni* with the presence of tree buttresses (Memmott 1991; Christensen and Vasquez 1982); and *L. rorotaensis* and *L. pessoana* were inversely associated with low cover (Rutledge and Ellenwood 1975a). Hence, although predictive risk maps based on regional determinants can identify geographical areas where a disease occurs or may occur, they cannot reliably identify precise locations of active transmission because of the focal nature of the vectors (Cross *et al.*, 1996). This is the rationale for identifying both regional and local environmental determinants of vector abundance.

For reasons outlined in Chapter 1, the study described in this chapter focuses specifically on the role of coffee plantations as a determinant of sandfly abundance and CL transmission. The evidence required to test whether coffee growing is a risk factor involves comparing sandfly vector abundance in forest, the apparent pristine habitat for sandflies in most of the sub-Andean region, with sandfly vector abundance in neighbouring coffee plantations. This comparison should take into account the strong environmental variability between coffee plantations, especially with respect to the method of cultivation (Chapter 1, section 1.3). The scarce studies carried out until now

have either compared only sandfly diversity and abundance in forest versus one type of coffee plantation (traditional coffee) (Warburg *et al.*, 1991), or they have compared sandfly diversity and abundance in different types of coffee plantations (traditional vs. intensified unshaded coffee growing) but did not examined forest (Alexander *et al.*, 2001; Alexander *et al.*, 2002). The studies reported to date strongly indicate that some sandfly species have adapted to traditional coffee plantations, but it remains unclear if any sandflies have adapted to intensive unshaded coffee plantations. If, as expected, adaptation of sandflies of the sub-Andean region to intensive unshaded coffee plantations is difficult (Chapter 1, section 1.3), the coffee intensification in the region may lead to a reduction in CL incidence (Alexander *et al.*, 2001). To examine the extent to which sandflies in Huila have adapted to coffee plantations, the studies described here compare sandfly communities in neighbouring locations, representing different types of coffee cultivation (at least the extreme types: traditional and intensive unshaded plantations) and forest. The study was designed to test whether the establishment of thriving sandfly populations in coffee plantations is solely due to the presence of coffee plants, or whether specific structural features are required (i.e. the presence of trees which resemble the original habitat, the sub-Andean forest, represented by traditional coffee plantations).

2.1.3 Outline and rationale

The present work describes the spatial distribution of the sandfly fauna in Huila department focussing on the identification of suspected sandfly vectors of CL. In addition, the study investigated potential regional and local ecological determinants for abundance of the two main sandfly species. Other local determinants for sandfly abundance related to the domestic environment, such as house features and potential hosts will be addressed in Chapter 3.

The study is described in seven main sections. The first section describes the sandfly fauna of the sub-Andean region of Huila department in relation to geography, altitude and habitat. The second section focuses on the abundance of the two main sandfly species identified in relation to geography. The third section explores the effect of location (height of the trap, position edge or centre within the habitat patch and location as indoors / outdoors) and type of trap on the catches of the two main sandfly species.

This section includes a validation of the catches by CDC traps as representative of the human landing catches. The fourth section concerns local determinants for abundance of the two main sandfly species. These include: a) general habitat type; b) habitat types according to physiognomic-structural classification and individual physiognomic-structural features; c) flora; d) other habitat features (i.e. protection from wind, slope, litter cover and depth in each habitat patch and distance to the nearest house). The fifth section concerns regional determinants for abundance of the two main sandfly species. These include: a) altitude; b) rainfall; c) temperature; d) soil; and e) slope of the general relief. The sixth section presents a multivariate analysis for all tested ecological determinants. The last section, investigates the geographical association between the abundance of the two main sandfly species and CL incidence in Huila.

2.1.4 Objectives

The overall goal of Chapter 2 is to describe quantitatively, and to identify determinants of, geographic variation in abundance of suspected sandfly vectors of cutaneous leishmaniasis (CL) in Huila department. The specific objectives are:

- 1) To describe the geographical distribution of sandfly species in the sub-Andean (coffee growing) region of Huila department (and compare with the distribution of reported CL cases).
- 2) To examine the role of regional ecological features such as climate (temperature, rainfall, altitude as a proxy) and physical factors (soil, slope) on the species composition and abundance of sandfly fauna, focusing on the two most abundant (*L. longiflocosa* and *L. nuneztovari*) anthropophilic species.
- 3) To examine the role of local ecological features such as physiognomy (evergreen, deciduous and semi-deciduous plants, and special life forms) and structure (e.g. strata number, height and cover) of the vegetation, flora, litter cover and depth, slope and degree of protection from wind on the species composition and abundance of sandfly fauna, focussing on the two most abundant anthropophilic species.
- 4) To evaluate the impact of forest replacement by coffee and the methods of coffee cultivation on the species composition and abundance of sandfly fauna, focussing on the anthropophilic species.

2.2 METHODS

2.2.1 Study sites

The survey area corresponds to the slopes of the sub-Andean Region (1000 – 2000 m a.s.l.) of Huila department (see Chapter 1, section 1.2), comprising an area of 7,800 km², 39.2% of the department (Figure 2.1) with a population of 98,395 inhabitants (12.7% of the department) (IGAC Instituto Geográfico Agustín Codazzi 1995). Seven municipalities were chosen (Figure 2.2), representing a range of climate, geographic features (Figures 1.4 and 1.6) and CL incidence [according to epidemiological records from 1982 – 1995, SSDH]: (1) Santa María [1 CL case reported], (2) Iquira [no cases]: both in the Cordillera Central in a zone with heavy annual rainfall (2000 - 2500 mm); (3) Garzón [no cases], (4), Algeciras [no cases], (5) Neiva [high incidence]: all in Cordillera Oriental in zone of medium rainfall (1200-2000 mm); (6) Baraya [high incidence]: in Cordillera Oriental in zone of lower rainfall (1000 - 1200 mm); and (7) Saladoblanco [1 case]: in Colombian Massif in medium rainfall zone. In each municipality a broad transect was defined, divided into three zones according to altitude: 1) At 2000 ± 200 m a.s.l.; 2) At 1500 ± 200 m a.s.l.; and 3) At 1000 ± 200 m a.s.l. . In each zone three to four sites were sampled simultaneously on two consecutive nights, recording the geographic coordinates with a Global Positioning System receiver (GPS 12, GARMIN®) and the altitude with an altimeter. Sampling sites were chosen according to the presence of the four habitats of interest: 1) forest, several types; 2) traditional coffee plantations, with moderate to heavy shade from trees; 3) intensive semishaded coffee plantations, with low shade from trees or other plants; and 4) intensive unshaded coffee plantations, where there was little or no shade (see Table 2.3).

A more precise description of each sampling site was achieved using Küchler's physiognomic-structural method for vegetation description (Küchler 1966). This is a hierarchical method, where ten main categories are formed based on life forms and leaf phenology (Table 2.2). Within the categories further differentiation of vegetation is achieved by the recording of height (eight categories), cover (six categories), leaf characteristics and the presence of other special life forms. Leaf phenology was recorded based on the bibliographic references for each identified species (plants with trunk diameter to the breast height, DBH, > 10 cm) within a representative transect (50 m x 4 m = 200 m²) for each sampling site (Annexe 1). Leaf phenology for plants with DBH less



Figure 2.1 Landscape of the sub-Andean Region of Huila department (Tello municipality, on the Cordillera Oriental). Photo by Raul Pardo.

Table 2.2 Symbols for physiognomic-structural description of vegetation (Küchler 1966, with some modifications by G. Paramo).

LIFE-FORM CATEGORIES			
BASIC LIFE FORMS		SPECIAL LIFE FORMS	
Woody plants		Climbers (lianas) ^a	C
Broad leaf evergreen	B	Stem succulents	K
Broad leaf deciduous	D	Tuft plants (e.g. palms and tree ferns)	T
Needleleaf evergreen	E	Bamboos	V
Needleleaf deciduous	N	Epiphytes ^a	X
Aphyllous	O		
Semideciduous (B + D)	S		
Mixed (D + E)	M	LEAF CHARACTERISTICS ^b	
Herbaceous plants		Hard (sclerophyll)	h
Graminoids	G	Soft	w
Forbs	H	Succulent	k
Lichens, mosses	L	Large (> 400 cm ²)	l
		Small (< 4 cm ²)	s
OTHER FEATURES			
Buttress root ^a	W		
Stilt root ^a	Z		
STRUCTURAL CATEGORIES			
Height		Coverage	
8 = 35 - 40 m		c = continuous (≥76%)	
7 = 20 - 35 m		i = interrupted (51 - 75%)	
6 = 10 - 20 m		p = parklike, in patches (26 - 50%)	
5 = 5 - 10 m		r = rare (6 - 25%)	
4 = 2 - 5 m		b = barely present, sporadic (1 - 5%)	
3 = 0.5 - 2 m		a = almost absent (< 1%)	
2 ^b = 0.1 - 0.5 m			
1 ^b = < 0.1 m			

^a Abundance was recorded in three categories based on the percentage of trees which had the special life form. ⁺ : low abundance (< 10%), ⁺⁺ : mid abundance (10 - 49%), and ⁺⁺⁺ : high abundance (> 50%); ^b These categories were not recorded.

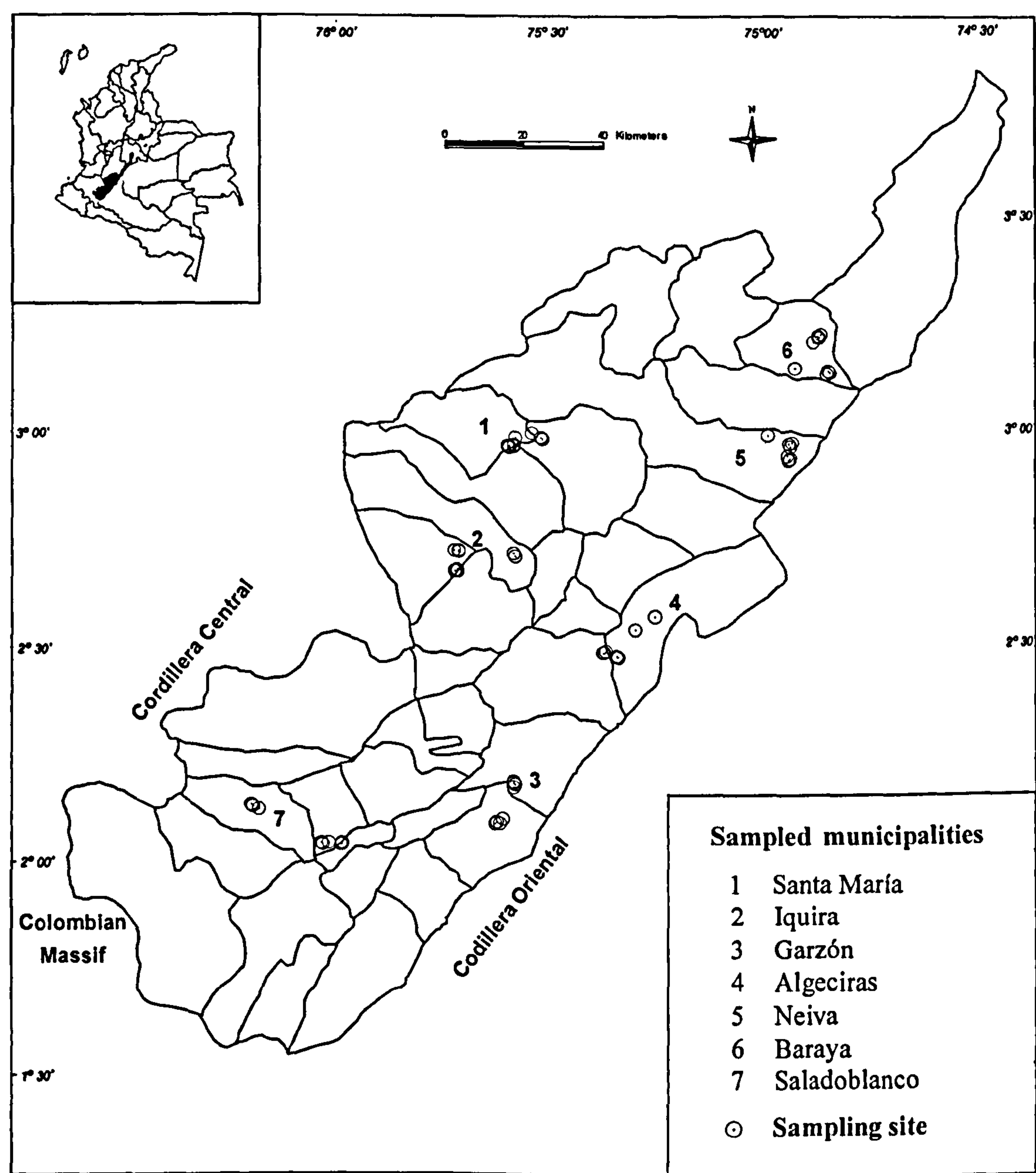


Figure 2.2 Sampling sites (n = 67) for the study of ecological determinants for sandflies in Huila department.

than 10 cm (height mainly < 5 m) was recorded only for common species (e.g. coffee, cacao and banana) or plant groups with well known phenology (e.g. palms and tree ferns).

Results are presented as formulae using letters and numbers which always follow the same sequence: 1) life form; 2) height categories; and 3) covering category for each height. This sequence applies to each recorded life form individually. Symbols for epiphytes, buttress roots and still roots are inserted after every height class where they are present. Symbol for lianas is inserted only in the upper most height class which they

reach. Within each formula, the most conspicuous feature (life form with higher cover) of the vegetation is placed at the beginning, with the exception of trees. A tree stratum is considered as the most important feature when it has cover higher than 50%, regardless of the higher cover by other lower strata.

Finally, collection and identification of plants were carried out as follows: for each plant, a sample of a branch with leaves, reproductive structures and fruits (if present) were collected and pressed (previous sprayed with alcohol 75%) recording the common name, site of collection, altitude, plant height, DBH and other features (e.g. flower colours, leaves smell and excretions). Samples were transported to the Herbario Nacional Colombiano (COL), in Bogotá where they were dried (24 to 48 h at 90°C approximately) prior to identification. Identification was carried out by Roberto Sánchez, BSc, MSc with the help of the plant collection of COL.

2.2.2 Sandfly sampling

Sandfly abundance was measured in all sampling sites using three methods: 1) CDC miniature light traps (LT) (Sudia and Chamberlain 1962); 2) human landing (HL); 3) direct aspiration of resting sandflies from tree trunks, only in forest and traditional coffee plantations (AT). LT were set at 1.5 m above the ground in both the centre and on the edge of each sampling site; and (when available) inside a house located in close proximity to each point (within 300 m radius). Additional traps were set in the centre and edge of the middle canopies (approximately 10 m high) of the forest and traditional coffee plantations. The traps were set all night (13 h, 18:00 – 07:00). HL catches were made for 40 minutes in the evenings (between 18:00 - 21:00) in some sampling sites. This time was selected arbitrarily. One protected person acted as bait and another collected the sandflies as soon as they landed*. These persons were members of the research team with large training in collections by HL. The AT was carried out by one person for 40 min during daylight, from soil level to 1.5 m above the ground. These collection methods were approved by the ethic committee of the National Institute of Health (INS). The collected sandflies were stored in vials containing 75% alcohol, and field information was recorded on a form (Annexe 2). Specimens were identified individually according to the keys of Young (1979), Young & Duncan (1994), with the help of the sandfly reference collection

* The participation of these persons was voluntary. They wore thick clothes and the person who acted as bait exposed only their forearms and lower legs.

of the INS in Bogotá. For each sandfly species some specimens were mounted in slides and they are held in the reference collection of the INS.

2.2.3 Quality of the sampling

Species richness in each region was estimated using the “sampled-base species accumulation curve method”, in order to check the extent to which the species sampled in each region reflected the full range of sandfly fauna. Sampled-base accumulation curves record the total number of sampled species revealed, during the process of data collection as additional sampled units are added to the pool of all previous collected samples (Gotelli and Colwell 2001). The unit of sampling effort was defined as the number of outdoor LT. Accumulation curves, for each region, were built as follows: 1) based on the raw data a smoothed curve was built using the EstimateS 7.5 software (Colwell 2005), 2) the asymptotic value of the accumulation curve obtained was estimated using the Clench equation (Soberón and Llorente 1993):

$$S_n = a.n / (1 + b.n)$$

where n is a measure of sampling effort, S_n is the predicted number of species at n , a represents the increased rate of species observation at the beginning of the sampling, b is a parameter related to the shape of the accumulation of new species during the sampling, and a / b represents the predicted asymptote (i.e. the predicted number of species). Sampling is considered “confident” if the slope $m [= a / (1 + b.n)^2] < 0.1$ (Jimenez-Valverde and Hortal 2003). Clench models are recommended for larger areas or for taxa for which the probability of adding a new species will improve as more time is spent in the field (Soberón 1993).

2.2.4 Explanatory variables for sandfly abundance

Twenty nine variables were examined as ecological determinants for sandfly abundance. Table 2.3 provides a short description of and the method for measuring each one. The variables are grouped in two main categories: local determinants (related to the micro-environment of each sampling site, recorded in situ) and regional determinants (related to the region, mainly recorded from maps). Regional determinants focused on climate (temperature and rainfall), soil type (thirteen classes), slope and altitude. Local determinants focused on distinguishing favourable habitats for sandflies including

Table 2.3 Variables which were examined as ecological determinants for sandfly abundance outdoors (as measured by CDC light traps).

Variable group	Name	Description	Measuring method	Tested as
Trap location	Municipality	One of the seven municipalities to which the sampling site belongs to: Santa Maria, Iquira, Saladoblanco, Garzon, Algeciras, Neiva, and Baraya.		Categorical
	CDC light trap location	Location of the CDC light traps on the edge and centre of each sampling site.	Categorized by direct observation	Binary
	CDC light trap height	Height of the CDC trap above the ground: 1.5 m and 10 m (only in forest and traditional coffee).	Categorized by direct observation	Binary
Local determinants	Habitat's types according to a general classification	Recording of four types of habitat: 1) forest. Several types; 2) traditional coffee plantations. Coffee plants with shade from trees (woody plants ≥ 5 m tall) moderate to heavy (covering > 50%); 3) intensive semishaded coffee plantations. This coffee growing type was defined where: (i) shade by trees was low (26 - 50% covering), or (ii) shade by trees was very low (< 25%), but the cover range mentioned in the item above was reached when cover by stratum 2 - 5 m tall (e.g. banana trees) was added, or (iii) shade provided by banana trees was high (covering > 76%); and 4) intensive unshaded coffee. This habitat was defined where : (i) there was little or not shade by trees and plants in the stratum 2 - 5 m tall (total covering ≤ 25%), or (ii) cover by banana trees was less or equal to 75% of the ground.	Categorized by direct observation	Categorical
	Habitat's types according to Kùchler's physiognomic-structural classification*	Detailed description of habitat according to physiognomic and structural features (Kùchler, 1966), grouped in 13 habitat categories (Table 2.14).	Description of each site by the Kùchler's formulae. Then, grouping sites based on similarities in physiognomic structural features using the software TWINSpan.	Categorical
	Slope	Main slope of the terrain in each sampling site, recorded in percentage (100% slope = 45°). Analysis based on eight ranges: 0 - 3, 3.1 - 7, 7.1 - 12, 12.1 - 25, 25.1 - 50, 50.1 - 75, 75.1 - 100, > 100.	Direct measurement with an Abney clinometer (Sokkia®, No. 8047-15)	Categorical
Number of tree strata	Litter cover	Percentage of cover by the litter within the transect, classified in four categories: 1) 20 - 40%, 2) 41 - 60%, 3) 61 - 80%, and 4) 81 -100%.	Direct observation	Categorical
	Litter depth	Litter depth (cm) was tested in three forms: a) depth of no decay litter, b) depth of partially decay litter, and c) depth of total litter.	Direct measurement with a ruler	Continuous
	Number of tree strata	A tree (woody plant > 5 m tall) stratum in a specific height category was recorded if trees, regardless of leaf phenology, had cover > 25%. Range: 0 - 4.	Taken from the Kùchler's formulae	Categorical

Table 2.3 Continued.

Variable group	Name	Description	Measuring method	Tested as
Leaf phenology	Leaf phenology	Dominant leaf phenology of the tree strata (excluding unshaded coffee where tree strata were absent). Classified in four classes: 1) deciduous, 2) semideciduous, 3) mixed of semideciduous and deciduous , and 4) mixed of semideciduous and evergreen.	Categorized taking into account the leaf phenology of the tree species in a given stratum.	Categorical
	Flora	Records of the presence of plant families within the transect. Eleven families were tested: Areaceae, Clusiaceae, Cyatheaceae, Fagaceae, Lauraceae, Mimosaceae, Moraceae, Musaceae (presence recorded when cover by this species was > 5%), Myrtaceae, Papilionaceae, and Rubiaceae.	Categorized after taxonomic identification of plants	Binary
	Cover	Percentage of the area of ground, within the transect, which was occupied by the above ground parts of each species (only trees > 5 m height).	Estimated by direct observation	Continuous
	Degree of protection from wind	The apparent protection from wind that relief features could provide. This variable was classified into three categories (see Table 2.4): a) protected, b) partially protected, c) unprotected.	Estimated by direct observation	Categorical
	Distance to the nearest house	Distance from the sampling site to the nearest house.	Estimated by direct observation	Continuous
Regional determinants	Altitude	Altitude (m a.s.l.) and altitude square of the locality where each sampling site was located.	Direct measure with an Altimeter (Thommen Classic®, TX-22, 6000m)	Continuous
	Temperature	Annual mean temperature (°C) and mean temperature square in each sampling site.	Read from map ^b	Continuous
	Rainfall	Total annual rainfall (mm) and annual rainfall square in each sampling site.	Read from map ^b	Continuous
	Soil type	Soil type, classified into thirteen classes: Mollic Hapludalfs - Typic Dystropepts (AQC), Entic Haplustolls - Typic Ustorthents - Lithic Ustorthents (LXA), Typic Humitropepts - Typic Troporthents - Typic Hapludands (MLB), Oxic Dystropepts - Typic Troporthents (MQA), Typic Humitropepts - Typic Hapludands (MQC), Typic Hapludolls - Typic Eutropepts - Typic Troporthents (MQD), Entic Hapludolls - Andic Humitropepts - Lithic Troporthents (MQE), Typic Humitropepts - Oxic Dystropepts (MQG), Fluvaquentic Eutropepts - Typic Tropofluvents - Fluventic Eutropepts (MQM), Lithic Ustorthents - Typic Haplustolls - Ustic Dystropepts (MRA), Typic Argiodolls - Typic Hapludalfs (PQA), Ustoxic Humitropepts - Entic Hapludolls - Typic Troporthents (PQF), Ustoxic Humitropepts - Ustic Dystropepts (PXG).	Read from map ^c	Categorical
Slope	Slope	Slope recorded in percentage (100% slope = 45°) and classified into six classes: 3.1 - 7, 7.1 - 12, 12.1 - 25, 25.1 - 50, 50.1 - 75, > 75.	Read from map ^c	Categorical

^a This variable was not included in the statistical analysis due to low number of replicates; ^b Mapa pluviométrico, Cuenca del alto Magdalena. Produced by IGAC, 1984, scale: 1:400.000; ^c Suelos, Departamento del Huila. Produced by IGAC, 1994; scale: 1:200.000.

different types of forest and coffee plantation, crops which have been circumstantially associated with sandflies (Chapter 1, section 1.3). As explained above, two approaches were applied to classify habitats: (1) a general classification of habitats into four classes (forest, and three types of coffee plantations); and (2) a refined classification based on Küchler's physiognomic-structural method, generating 13 habitat groups (see section 2.2.6). Some physiognomic structural features were tested separately (number of tree strata, leaf phenology, flora and cover). Further site features recorded and tested included: degree of protection from wind, slope, litter cover and depth, and distance to the nearest house. Degree of protection from wind was indirectly estimated on the basis of relief (general landform of the terrain surrounding each sampling site) and microrelief (relief features within each sampling site) (Table 2.4). Canyon areas were considered as a protective relief as most canyons within the study area are located transverse to the direction of the major winds (North-East winds). Other relief types (e.g. top of mountain, mountainside, valley, and hillside) were considered as unprotected. Regarding microrelief, "v" shapes and concave depressions were considered as wind protected. Flat or inclined terrains were considered as unprotected. Based on the combination of these relief and microrelief features, the sampling sites were classified according to three categories: (1) protected, when both relief and microrelief types were protective; (2) partially protected, when only one of the two feature was protective; and (3) unprotected, when both features were not protective (Table 2.4).

2.2.5 Geographical association between sandfly species and CL incidence

The geographical association between the two main anthropophilic/anthropophagic sandfly species and CL incidence was addressed by inspection of the spatial distribution of outdoor sandfly abundance (as measured by LTC) recorded during the study in 1998 and mean annual incidence of CL (records 1982 – 2004, SSDH) by municipality, both mapped using the software Arc View 3.1 (Environmental System Research Institute 1992-1998). The maps of CL incidence excluded (i) the period 1996 – 1999, where records at municipality level were not available and (ii) data from the 15 municipalities with only one or two cases (total cases = 24) within the 19 years of study period because it was considered that these cases could not be autochthonous (probably imported from other municipalities).

Table 2.4 Definition of categories of degree of protection (based on relief features) from wind used to classify each sampling site.

Relief		Microrelief	
Protected site (A)	Unprotected site (B)	Protected site (a)	Unprotected site (b)
mountain side on a canyon	mountain side	"v" shape depression	flat terrain (0 - 19% slope)
river bank on a canyon	top of mountain	concave depression	inclined terrain (≥ 20% slope)
	valley		
	river bank in valley		
	hillside		
	foothill side		

Feature combinations	Category of protection
Aa	protected
Ab, Ba	partially protected
Bb	unprotected

2.2.6 Statistical analysis

The analysis focuses on the two dominant species, *L. longiflocosa* and *L. nuneztovari*, which accounted for 89.2% and 4.6%, respectively, of all sandflies caught by all collection methods. An exception was the comparison of habitats by sandfly species where all anthropophilic sandfly species were included. Due to the high overdispersion of the sandfly data (LT outdoors, in the majority of cases), catches are presented as Williams’ geometric means (GM) (William 1937), including their 95% confidence intervals, using the transformation $\ln(x + 1)$, except where stated. In order to identify the ecological determinants for the two major sandfly species, univariate and multivariate statistical analyses were carried out for each species using Generalized Linear Models (GLIM) (Crawley 1993) and Stata 7 software (Stata Corporation 2001). *L. longiflocosa* models made the assumption that sandfly counts, followed a negative binomial distribution. Most analyses excluded the two municipalities where this species was absent, with the exception of models testing the effect of either rainfall or temperature (as these parameters were suspected a priori of being responsible for these absences). *L. nuneztovari* models assumed a normal distribution of errors, following a log transformation of the raw data, $\ln(x + 1)$. Univariate analysis was carried out on each of

the possible ecological determinants controlling for general habitat types, trap position and height, clustering by sample site to generate robust standard errors. For multivariate analysis, a Maximum Model, including all possible explanatory variables (ecological determinants) was generated. Then, the model was simplified by backward elimination of the least significant explanatory variables using the Wald test or F ratio test, whichever was appropriate. Finally, a Minimum Adequate Model (MAM) was obtained where all the explanatory variables were significant ($p \leq 0.05$). The analyses presented for *L. longiflocosa* exclude the outlier (5,432 sandflies in a single LT); although when a significant association was detected, the robustness of this association was checked by repeating the models in the presence of the outlier. Validation of the MAMs was carried out by checking the appropriate residuals plots and the normality of residuals (Quantile-Quantile plot).

Grouping of habitats described according to the K  chler’s physiognomic-structural method was carried out using “Two Ways Indicator Species Analysis” or TWINSpan (Hill 1979), a program used in ecology for classifying plants species data in samples. The analysis is based on progressive refinement of a single axis ordination from reciprocal averaging analysis (Kent and Coker 1994). Although the technique is based on presence/absence, quantitative data (i.e. abundance) can be incorporated by considering different abundance levels of the same species to be different species (pseudospecies). The final output is shown in a two-ways table (e.g. Annexe 3) where at the bottom, in several rows, is the dichotomized key for sites which shows both the group structure and the sequence of division. Taking the first row (first division of sites), the sites are split into two groups: one formed by 39 zeros (first group) and another by 18 ones (second group). Each of these groups is split again into two groups as indicated by the second row (second division of sites). So, the second group of the first row is split into 15 zeros and three ones and so on. Interpretation of the table is subjective. Groups can be taken from different levels, but they should make ecological sense. The ecological basis for re-grouping of sites in this study was similarities in tree strata number. For the analysis of the data from K  chler’s physiognomic-structural classification, the formula describing each sampling site was split into each life form component of each height stratum, ignoring the cover data. Then, each life form component was taken as a “species” for the analysis. Data on percentage of coverage for each life form component was added as categories of abundance (Table 2.2) for each of

the “species”, changing the letters a, b, r, p, i, and c by the numbers 1, 2, 3, 4, 5, 6, respectively.

Comparison of general habitat types (forest and coffee plantations) was also made by measuring beta (β) and gamma (γ) diversity, in relation to the complete anthropophilic sandfly fauna. β biodiversity is defined as the difference in species diversity between habitats, and depends on the number of species that are unique to each of the habitats being compared. γ biodiversity is a measure of the overall diversity from the different habitats within a region (Harrison *et al.*, 2004). Males of some low abundance species, where females were not distinguished, were joined to the subgenus where the females presumably belong to (i.e. *L. erwindonaldi*, *L. scorzai*, and *L. sp. of pichinde* were joined to *L. (Helcocyrtomyia) spp.*; and *L. leray* were joined to *L. (Psathyromyia) spp.*). Analyses reflected presence of the selected species for all collection methods, excluding LT indoors (because a proportion of houses were located outside the study habitats), and excluded the seven sampled sites with no sandflies. A cluster analysis technique was used to classify the sites and test for any consistent pattern of habitats clumping according to the sandfly fauna (as measured by outdoor LTC). This method proceeds from individual samples and progressively combines them in terms of their similarity until all samples are in one group. Similarity between sites was measured using the Percentage Similarity Index or Renkonen Index (Krebs 1999):

$$P = \sum_i \text{minimum}(p_{1i}, p_{2i})$$

Where P = Percentage similarity between sample 1 and 2; p_{1i} = percentage of species i in community sample 1; p_{2i} = percentage of species i in community sample 2.

Similarity Index is calculated in two steps: (i) by expressing the abundance of the different species as percentages in each of the two sampled sites to be compared, which must sum to 100%; and (ii) by summing the minimum percentage for each species. Percentage Similarity Index is a quantitative index which reflects the relative abundance of anthropophilic sandfly species. The index was selected because it is relatively unaffected by sample size and species diversity. The index ranges from 0 (no similarity) to 100 (complete similarity). Clustering was achieved by the un-weighted pair-group method using arithmetic averages (UPGMA), carried out within the software MVSP 3.1 (Kovach Computing Services 1985-2004), and illustrated as a dendrogram (Krebs 1999).

In order to interpret any perceived geographical relationship between *L. longiflocosa* LTC abundance and CL incidence, it was necessary to validate the role of LTC as a quantitative indicator of HLC (the true reflection of human sandfly exposure). Two areas where *L. longiflocosa* was absent were excluded. The relationship between the log transformed abundance (+1) measurements from the two sampling methods was evaluated using the correlation coefficient (r), using t test to test for significance.

2.3 RESULTS

2.3.1 Sandfly fauna in Huila in relation to geography, altitude and habitat

The fieldwork was carried out between March and August 1998. A total of 31,693 sandflies were collected from 67 different sites in 715 samples (Table 2.5). At least 21 sandfly species, including at least 13 anthropophilic species, were identified. The most abundant sandfly was *L. longiflocosa* Osorno-Mesa, Morales, Osorno & Hoyos, 1970 which accounted for 89.2% (28,267 specimens) of all sandflies collected and was the dominant species for each sampling method. The second species was *L. nuneztovari* (Ortiz, 1954) with 4.6% (1,465 specimens). The remaining 5.7% (1,804 specimens) included approximately 19 species, of which 11 are known as anthropophilic: *L. andina* Osorno, Osorno-Mesa & Morales, 1972 (57 specimens), *L. lichyi* (Floch & Abonnenc, 1950) ($n = 52$), *L. columbiana* (Ristorcelli & Van Ty, 1941) ($n = 38$), *L. scorzai* (Ortiz, 1965) ($n = 30$), *L. longipalpis* (Lutz & Neiva, 1912) ($n = 24$), *L. ayrozai* (Barretto & Coutinho, 1940) (12 specimens), *L. erwindonaldi* (Ortiz, 1978) ($n = 9$), *L. gomezi* (Nitzulescu, 1931) ($n = 8$), *L. lerayi* Le Pont, Martinez, Torrez-Espejo & Dujardin, 1998 ($n = 8$), *L. oresbia* (Fairchild & Hertig, 1961) ($n = 6$), and *L. pia* (Fairchild & Hertig, 1961) ($n = 4$). Some unidentified specimens, mostly females which did not fit into the described species in the keys, belonged to the subgenera *Helcocyrtomyia* ($n = 286$) and *Psathyromyia* ($n = 8$) were also considered anthropophilic - based on the human landing catches. A total of 0.5% ($n = 57$) of sandflies, were unidentified due to damage.

2.3.1.1 Distribution by geography

Sandfly diversity was greatest in the municipalities located on Cordillera Oriental (at least 19 species found), followed by Cordillera Central (at least 11 species) and the Colombian Massif (at least seven species) (Table 2.6). According to the Clench richness

Table 2.5 Sandfly species composition and relative abundance by collection method in Huila department. The four most abundant species and their totals are highlighted in bold for each sampled method (n = number of catches).

Lutzomyia species	CDC outdoors (n = 459)						CDC indoors (n = 108)						Human Landing (n = 103)						Resting on tree trunk (n = 45)						Total (n = 715)					
	Female			Total			Female			Total			Female			Total			Female			Total			Female			Total		
		%		%		%		%		%		%		%		%		%		%		%		%		%		%		
L. andina^a	17	0.1	23	0.1	6	0.7	9	1.0	6	0.1	6	0.1	6	0.1	1	0.2	19	2.4	30	0.1	57	0.2								
L. atroclavata	9	0.1	15	0.1	9	1.0	10	1.1	0	0	0	0	0	0	0	0	2	0.3	18	0.1	27	0.1								
L. ayrozai ^a	4	<0.1	11	0.1	0	0	0	0	1	<0.1	1	<0.1	1	<0.1	0	0	0	0	5	<0.1	12	<0.1								
L. carpenteri	223	1.4	632	3.1	0	0	2	0.2	0	0	0	0	0	0	0	0	0	0	223	0.9	634	2.0								
L. columbiana ^a	24	0.2	24	0.1	4	0.5	4	0.4	10	0.1	10	0.1	10	0.1	0	0	0	0	38	0.2	38	0.1								
L. dubitans	22	0.1	32	0.2	7	0.8	13	1.4	0	0	0	0	0	0	0	0	0	0	29	0.1	45	0.1								
L. erwindonaldoi ^a	0	0	5	<0.1	0	0	0	0	1	<0.1	4	<0.1	4	<0.1	0	0	0	0	1	<0.1	9	<0.1								
L. gomezi ^a	4	<0.1	5	<0.1	1	0.1	1	0.1	2	<0.1	2	<0.1	2	<0.1	0	0	0	0	7	<0.1	8	<0.1								
L. (Helcocyrtomyia) spp.^a	108	0.7	114	0.6	6	0.7	6	0.6	166	2.0	166	1.8	166	1.8	0	0	0	0	280	1.1	286	0.9								
L. (Psathyromyia) spp. ^{*a}	6	<0.1	6	<0.1	0	0	0	0	2	<0.1	2	<0.1	2	<0.1	0	0	0	0	8	<0.1	8	<0.1								
L. lerayi ^a	0	0	7	<0.1	0	0	0	0	0	0	1	<0.1	1	<0.1	0	0	0	0	0	0	8	<0.1								
L. lichyi ^a	34	0.2	34	0.2	3	0.3	3	0.3	12	0.1	12	0.1	12	0.1	2	0.4	3	0.4	51	0.2	52	0.2								
L. longiflocosa^a	14346	91.2	17937	87.1	711	82.4	746	79.5	7874	95.9	9013	96.2	474	92.9	571	71.4	23405	92.5	28267	89.2	89.2	89.2								
L. longipalpis ^a	8	0.1	18	0.1	1	0.1	6	0.6	0	0	0	0	0	0	0	0	0	0	9	<0.1	24	0.1								
L. nuneztovari^a	598	3.8	1233	6.0	78	9.0	89	9.5	130	1.6	130	1.4	3	0.6	13	1.6	809	3.2	1465	4.6	4.6	4.6								
L. oresbia ^a	2	<0.1	3	<0.1	2	0.2	2	0.2	1	<0.1	1	<0.1	1	<0.1	0	0	0	0	5	<0.1	6	<0.1								
L. pia ^a	2	<0.1	3	<0.1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1	2	<0.1	4	<0.1								
L. pilosa	1	<0.1	1	<0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	<0.1	1	<0.1								
L. punctigeniculata	1	<0.1	1	<0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	<0.1	1	<0.1								
L. shannoni	0	0	8	<0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	<0.1	<0.1	<0.1							
L. trinidadensis	243	1.5	323	1.6	24	2.8	29	3.1	0	0	0	0	0	0	30	5.9	190	23.8	297	1.2	542	1.7								
L. scorzai ^a	0	0	23	0.1	0	0	0	0	0	0	7	0.1	7	0.1	0	0	0	0	0	30	0.1	0.1	0.1							
L. sp. of pichinde	0	0	4	<0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	<0.1	<0.1	<0.1							
Unidentified	74	0.5	125	0.6	11	1.3	18	1.9	8	0.1	13	0.1	13	0.1	0	0	1	0.1	93	0.4	157	0.5								
Total	15726	20587	863	938	8213	9368	510	800	25312	31693	9368	9368	510	800	25312	31693	9368	9368	25312	31693	31693	31693								

* Undistinguished females. Mixed of *L. shannoni* and *L. lerayi*; ^a Anthropophilic or opportunistic human biter sandfly.

Table 2.6 Sandfly species composition and relative abundance by geographical region and municipality in Huila department (all collection methods). The four most abundant species and their totals are highlighted in bold for each sampled municipality.

Lutzomyia species	Cordillera Central				Colombian Massif				Cordillera Oriental													
	Santa María (n = 89)		Iquira (n = 87)		Sanladoblanco (n = 98)		Garzón (n = 121)		Algeciras (n = 101)		Neiva (n = 114)		Baraya (n = 105)									
	Female	% Total	% Female	% Total	Female	% Total	% Female	% Total	Female	% Total	% Female	% Total	Female	% Total								
Lutzomyia species	Female	% Total	% Female	% Total	Female	% Total	% Female	% Total	Female	% Total	% Female	% Total	Female	% Total								
<i>L. andina</i> ^a	0	0	0	0	0	0	20	3.9	3.4	1	<0.1	3	<0.1	5	0.2	17	0.5	4	<0.1	4	<0.1	
<i>L. atroclavata</i>	16	2.5	2	2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	<0.1	
<i>L. ayrozai</i> ^a	0	0	0	0	5	2.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>L. carpenteri</i>	0	0	4	4.9	7	5.1	8	1.6	1.5	206	2.9	604	5.9	0	0	0	0	5	<0.1	8	0.1	
<i>L. columbiana</i> ^a	0	0	0	0	0	0	23	4.5	2.3	0	0	0	0	5	0.2	5	0.2	10	0.1	10	0.1	
<i>L. dubitans</i>	4	0.6	6	7.4	1	0.5	7	1.4	1.1	3	<0.1	6	0.1	2	0.1	4	0.1	6	<0.1	7	<0.1	
<i>L. erwindonaldoi</i> ^a	1	0.2	1	0.7	0	1	0	0	0	0	0	1	<0.1	0	0	4	0.1	0	0	1	<0.1	
<i>L. gomezi</i> ^a	0	0	0	0	0	0	0	0	0	7	0.1	8	0.1	0	0	0	0	0	0	0	0	
<i>L. (Helcocyrtomyia) spp.</i> ^a	2	0.3	3	11.1	8	3.9	16	3.1	1.6	26	0.4	26	0.3	173	6.7	177	5.6	46	0.3	46	0.3	
<i>L. (Psathyromyia) spp.</i> ^a	0	0	0	0	0	0	4	0.8	0.4	4	0.1	4	<0.1	0	0	0	0	0	0	0	0	
<i>L. lerayi</i> ^a	0	0	0	0	0	0	0	7	0.7	0	0	1	<0.1	0	0	0	0	0	0	0	0	
<i>L. lichi</i> ^a	3	0.5	2	2.5	14	6.8	32	6.2	3.3	0	0	0	0	0	0	0	0	0	0	0	0	
<i>L. longiflocosa</i> ^a	379	58.4	0	0	0	0	62	12.1	10.4	6860	95.4	9420	92.2	2252	87.1	2701	85.0	13852	98.4	15399	97.6	
<i>L. longipalpis</i> ^a	0	0	3	3.7	12	8.8	0	0	0	1	<0.1	6	0.1	4	0.2	5	0.2	1	<0.1	1	<0.1	
<i>L. nuneztovari</i> ^a	122	18.8	34	42.0	38	27.9	336	65.5	71.7	33	0.5	68	0.7	75	2.9	94	3.0	66	0.5	75	0.5	
<i>L. oresbia</i> ^a	0	0	0	0	0	0	2	0.4	0.3	0	0	0	0	2	0.1	2	0.1	0	0	0	0	
<i>L. pia</i> ^a	0	0	0	0	0	0	0	0	0	0	0	2	<0.1	2	0.1	2	0.1	0	0	0	0	
<i>L. pilosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	<0.1	1	<0.1	0	0	0	0	
<i>L. punctigeniculata</i>	0	0	1	1.2	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>L. shannoni</i>	0	0	0	0	0	0	0	0	0.8	0	0	0	0	0	0	0	0	0	0	0	0	
<i>L. trinidadensis</i>	121	18.6	18	22.2	48	35.3	31	15.0	43	15.5	4	0.1	4	<0.1	55	2.1	141	4.4	68	0.5	139	0.9
<i>L. scorzai</i> ^a	0	0	0	2	1.5	0	0	5	0.5	3	<0.1	3	<0.1	0	0	9	0.3	0	0	11	0.1	
<i>L. sp. of pichinde</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0.1	0	0	0	0	
Unidentified	1	0.2	2	2.5	2	1.5	3	0.6	0.8	49	0.7	57	0.6	9	0.3	11	0.3	26	0.2	72	0.5	
total	649	1131	81	136	206	278	513	984	984	7194	10213	2585	3177	14084	15774							

^a Anthrophilic or opportunistic human biter sandfly.

estimator, based on outdoor LT catches, the percentage of collected species was high: Cordillera Oriental, 92.1% (20 / 21.71); Cordillera Central, 89.9% (12 / 13.35); and Colombian Massif, 91.9% (7 / 7.62), (Table 2.7, Annexe 4). The slope indicates also that the sampled sandfly fauna was representative of the total fauna in each region ($m < 0.1$). Each of the three models explained a high percentage of the variance ($r^2 > 89\%$).

Anthropophilic sandfly species were also more diverse in the municipalities of Cordillera Oriental where 11 out of 12 anthropophilic species were found (exception was *L. ayrozai*); in the Cordillera Central there were at least six anthropophilic species (*L. longiflocosa*, *L. nuneztovari*, *L. lichyi*, *L. longipalpis*, and *L. (Helcocyrtomyia) spp.*, including *L. erwindonaldi* and *L. scorzai*); and in the Colombian Massif there were five (*L. nuneztovari*, *L. lichyi*, *L. oresbia*, *L. ayrozai*, and *L. (Helcocyrtomyia) spp.*, including *L. erwindonaldi*). Five sandfly species were widely distributed: *L. nuneztovari*, *L. dubitans* and *L. (Helcocyrtomyia) spp.* were found in all seven municipalities; *L. erwindonaldi* in six (excepting Garzón); and *L. longiflocosa* in five municipalities (with the exception of Iquira and Saladoblanco). Some species were unique to a single region: Cordillera Oriental (eight sandflies species): *L. andina* and *L. columbiana*, *L. gomezi*, *L. pia*, *Psathyromyia spp.* (including *L. lerayi* and *L. shannoni*), *L. pilosa* and *L. sp. of pichinde*; Cordillera Central (one species): *L. punctigeniculata*; and Colombian Massif (one species): *L. ayrozai*.

In the Cordillera Oriental *L. longiflocosa* was the most abundant species in Baraya (98% of total), Algeciras (92%) and Neiva (85%), but *L. nuneztovari* was the most dominant, 73%, in Garzón. In the Cordillera Central, *L. longiflocosa* dominated in Santa María (57%), while, in Iquira *L. trinidadensis*, 35%, and *L. nuneztovari*, 28%, shared dominance. In the Colombian Massif (Saladoblanco), *L. nuneztovari* dominated (70%).

2.3.1.2 Distribution by altitude

Sandfly species diversity was similar in the three altitudinal ranges: low (900 – 1299 m a.s.l.): 12 species; medium (1300 – 1699 m a.s.l.): 10 species; and high (1700 – 2100 m a.s.l.): 11 species (Table 2.8). There was some suggestion that anthropophilic sandfly

Table 2.7 Results of Clench species richness estimator and observed species richness for each sampled region according to catches by CDC traps outdoors.

Equation parameters and results ^a	Cordillera Central	Colombian Massif	Cordillera Oriental
<i>n</i>	120	62	277
<i>a</i>	0.6689	0.9155	0.9258
<i>b</i>	0.0501	0.1202	0.0426
<i>m</i> (slope)	0.01	0.01	0.006
<i>r</i> ²	98.2	98.5	99.4
<i>S</i> _{obs} (Observed number of species)	12	7	20
<i>S</i> _{pr} (predicted number of species, <i>a</i> / <i>b</i>)	13.4	7.6	21.7
Percentage of collected species ([<i>S</i> _{obs} / <i>S</i> _{pr}] x100)	89.9	91.9	92.1

^a See section 2.2.3 for detailed explanation.

species diversity was lower at low altitude (*n* = 6), compared to medium (8) or high altitude (9). Only three species, *L. longiflocosa*, *L. nuneztovari* and *L. (Helcocyrtomyia) spp.*, were distributed in all three altitudinal ranges. Five species were present only in the low altitudinal range: *L. gomezi*, *L. atroclavata*, *L. pilosa*, *L. punctigeniculata*, and *L. carpenteri*; and five were present only in the highest altitudinal range: *L. ayrozai*, *L. oresbia*, *L. pia*, *L. lerayi* and *L. shannoni*. *L. longiflocosa* dominated the sandfly fauna at medium (95%) and high altitude (86%) sample sites, but at low altitude (900 – 1299 m a.s.l.) the dominant species were *L. carpenteri*, 39% and *L. trinidadensis*, 32%.

2.3.1.3 Distribution by habitat

Sandfly diversity was highest in forest (20 species), followed by traditional coffee (11 species), semishaded coffee (10 species), and unshaded coffee (8 species) (Table 2.9). *L. longiflocosa* was the dominant species in all habitats (90%) except semishaded coffee where *L. nuneztovari* dominated (58%) (Table 2.9). Diversity of anthropophilic sandfly species was also highest in forest (12 species, excepting *L. gomezi*), compared with traditional coffee (7 species); semishaded coffee (6 species) and unshaded coffee (6 species). Comparison of habitats by β diversity of anthropophilic sandfly species

Table 2.8 Sandfly species composition and relative abundance by altitudinal ranges in Huila department (all collection methods). The four most abundant species and its totals are highlighted in bold for each altitudinal range.

Lutzomyia species	Altitudinal range (m a.s.l.)											
	900 - 1299				1300 - 1699				1700 - 2100			
	(n = 206)				(n = 274)				(n = 235)			
	Female	%	Total	%	Female	%	Total	%	Female	%	Total	%
<i>L. andina</i> ^a	0		0		1	<0.1	1	<0.1	29	0.7	56	1.1
<i>L. atroclavata</i>	18	2.1	27	1.7	0		0		0		0	
<i>L. ayrozai</i> ^a	0		0		0		0		5	0.1	12	0.2
<i>L. carpenteri</i>	223	25.6	634	39.0	0		0		0		0	
<i>L. columbiana</i> ^a	0		0		9	<0.1	9	<0.1	29	0.7	29	0.6
<i>L. dubitans</i>	27	3.1	41	2.5	2	<0.1	4	<0.1	0		0	
<i>L. erwindonaldoi</i> ^a	1	0.1	7	0.4	0		2	<0.1	0		0	
<i>L. gomezi</i> ^a	7	0.8	8	0.5	0		0		0		0	
<i>L. (Helcocyrtomyia) spp.</i> ^a	98	11.3	98	6.0	90	0.4	91	0.4	92	2.2	97	2.0
<i>L. (Psathyromyia) spp.</i> ^a	0		0		0		0		8	0.2	8	0.2
<i>L. lerayi</i> ^a	0		0		0		0		0		8	0.2
<i>L. lichyi</i> ^a	46	5.3	47	2.9	5	<0.1	5	<0.1	0		0	
<i>L. longiflocosa</i> ^a	31	3.6	34	2.1	19662	96.9	24003	95.4	3712	89.6	4230	86.4
<i>L. longipalpis</i> ^a	8	0.9	23	1.4	1	<0.1	1	<0.1	0		0	
<i>L. nuneztovari</i> ^a	123	14.1	181	11.1	436	2.1	885	3.5	250	6.0	399	8.1
<i>L. oresbia</i> ^a	0		0		0		0		5	0.1	6	0.1
<i>L. pia</i> ^a	0		0		0		0		2	<0.1	4	0.1
<i>L. pilosa</i>	1	0.1	1	0.1	0		0		0		0	
<i>L. punctigeniculata</i>	1	0.1	1	0.1	0		0		0		0	
<i>L. shannoni</i>	0		0		0		0		0		8	0.2
<i>L. trinidadensis</i>	283	32.5	519	31.9	14	0.1	23	0.1	0		0	
<i>L. scorzai</i> ^a	0		0		0		19	0.1	0		11	0.2
<i>L. sp. of pichinde</i>	0		0		0		0		0		4	0.1
Unidentified	3	0.3	5	0.3	79	0.4	127	0.5	11	0.3	25	0.5
Total	870		1626		20299		25170		4143		4897	

^a Anthrophilic or opportunistic human biter sandfly.

showed high variability between forest and either of the three coffee habitats (β ranged from 6 - 7) and confirmed that forest was the most diverse habitat (Table 2.10). Forest sites had six anthropophilic species not found in traditional coffee, semishaded coffee or unshaded coffee. Only one species found in (traditional) coffee was not found in forest. There were few differences between coffee types (β range 1-3), notably *L. gomezi* being

Table 2.9 Sandfly species composition and relative abundance by habitat type in Huila department (all collection methods, except indoor CDC traps, were included). The four most abundant species and its totals are highlighted in bold for each sampled method.

Lutzomyia species	Habitat											
	Forest (n = 292)			Traditional coffee (n = 117)			Intensive semishaded coffee (n = 105)			Intensive unshaded coffee (n = 93)		
	Female	%	Total	Female	%	Total	Female	%	Total	Female	%	Total
L. andina ^a	24	0.1	48	0		0	0		0	0		0
L. atroclavata	1	<0.1	3	6	0.1	10	1	0.4	1	1	0.1	3
L. ayrozai ^a	5	<0.1	12	0		0	0		0	0		0
L. carpenteri	210	1.2	600	10	0.2	26	3	1.1	6	0		0
L. columbiana ^a	32	0.2	32	0		0	0		0	2	0.1	2
L. dubitans	7	<0.1	10	7	0.1	10	3	1.1	5	5	0.3	7
L. erwindonaldoi ^a	0		3	1	<0.1	5	0		1	0		0
L. gomezi ^a	0		0	6	0.1	7	0		0	0		0
L. (Helcocyrtomyia) spp. ^a	145	0.8	151	67	1.3	67	5	1.9	5	57	3.8	57
L. (Psathyromyia) spp. ^a	8	<0.1	8	0		0	0		0	0		0
L. lerayi ^a	0		8	0		0	0		0	0		0
L. lichyi ^a	29	0.2	29	4	0.1	4	13	4.9	14	2	0.1	2
L. longiflocosa ^a	16354	93.9	19941	4846	92.1	5783	124	47.1	143	1370	91.0	1654
L. longipalpis ^a	1	<0.1	2	4	0.1	4	3	1.1	12	0		0
L. nunezovari ^a	494	2.8	866	101	1.9	140	88	33.5	305	48	3.2	65
L. oresbia ^a	3	<0.1	4	0		0	0		0	0		0
L. pia ^a	2	<0.1	4	0		0	0		0	0		0
L. pilosa	1	<0.1	1	0		0	0		0	0		0
L. punctigeniculata	1	<0.1	1	0		0	0		0	0		0
L. shannoni	0		8	0		0	0		0	0		0
L. trinidadensis	52	0.3	143	182	3.5	311	21	8.0	29	18	1.2	30
L. scorzai ^a	0		19	0		4	0		4	0		3
L. sp. of pichinde	0		4	0		0	0		0	0		0
Unidentified	48	0.3	86	29	0.6	44	2	0.8	4	3	0.2	5
Total	17417		21983	5263		6415	263		529	1506		1828

^a Anthropophilic or opportunistic human biter sandfly.

Table 2.10 Presence / absence of anthropophilic sandfly species by habitat type and sampling site (all collections methods, except CDC traps indoors).

Habitat and site code ^a	<i>Lutzomyia</i> species												
	<i>L. andina</i>	<i>L. ayrozal</i>	<i>L. columblana</i>	<i>L. gomezi</i>	<i>L. lichyi</i>	<i>L. longifocosa</i>	<i>L. longipalpis</i>	<i>L. nuneztovari</i>	<i>L. oresbla</i>	<i>L. pia</i>	<i>Helcocyrtomyia</i> spp.	<i>Psathyromyia</i> spp.	Total species
Forest (n = 24) ^b													
AL40fo	x				x		x			x	x		5
AL44fo					x		x			x			3
AL45fo					x				x				2
AL47fo					x		x						2
BA57fo					x		x			x			3
BA58fo	x				x					x			3
BA60fo	x		x		x		x			x			5
BA63fo	x		x		x		x			x			5
GA31fo	x		x		x		x	x		x	x		7
GA32fo	x		x		x		x			x	x		6
GA34fo			x		x		x			x			4
GA36fo				x	x		x						3
IQ10fo							x			x			2
IQ13fo				x						x			2
IQ15fo							x						1
NE48fo					x		x			x			3
NE49fo	x		x		x		x	x	x	x			7
NE50fo	x				x		x		x	x			5
NE56fo					x	x				x			3
SA22fo		x								x			2
SA24fo		x					x	x		x			4
SA28fo				x			x						2
SM6fo					x		x			x			3
SM8fo					x		x						2
Total positive sites	8	2	6	0	3	18	1	19	3	3	18	3	11
Traditional coffee (n = 11)													
AL39tc			x		x		x						3
AL42tc					x		x			x			3
BA61tc										x			1
BA64tc					x		x			x			3
BA65tc					x	x				x			3
NE53tc					x		x			x			3
NE55tc					x	x	x			x			4
SA25tc				x			x						2
SM1tc					x		x						2
SM3tc				x	x		x			x			4
SM9tc					x		x						2
Total positive sites	0	0	0	1	2	9	2	9	0	0	7	0	6

Table 2.10 Continued.

Lutzomyia species Habitat and site code*		Lutzomyia species												
		L. andina	L. ayrozal	L. columbiana	L. gomezi	L. lichyl	L. longiflucosa	L. longipalpis	L. nuneztovari	L. oresbia	L. pla	Helcocyrtomyia spp.	Psathyromyia spp.	Total species
Semishaded coffee (n = 10)														
BA59sc						x								1
GA29sc					x			x						2
GA35sc					x	x		x			x			4
GA37sc					x			x						2
GA38sc								x						1
IQ14sc					x		x							2
SA20sc					x			x						2
SA26sc					x			x			x			3
SA27sc					x			x						2
SM5sc						x		x						2
Total positive sites		0	0	0	0	7	3	1	8	0	0	2	0	5
Unshaded coffee (n = 15) ^c														
AL41uc						x								1
AL43uc						x		x			x			3
AL46uc						x		x						2
BA66uc						x								1
BA67uc						x								1
GA30uc					x			x			x			3
GA33uc				x		x		x						3
IQ11uc								x						1
IQ12uc								x						1
NE51uc						x					x			2
NE52uc						x		x			x			3
NE54uc						x		x			x			3
SA21uc					x			x						2
SM4uc						x		x						2
SM7uc						x		x						2
Total positive sites		0	0	1	0	2	11	0	11	0	0	5	0	5
β diversity														
Compared habitats		# of species unique for each habitat				β								
		fo	tc	sc	uc									
Forest vs. traditional coffee		6	1			7								
Forest vs. semishaded coffee		6		0		6								
Forest vs. unshaded coffee		6			0	6								
Traditional coffee vs. semishaded coffee			1	0		1								
Traditional coffee vs. unshaded coffee			2		1	3								
Semishaded coffee vs. unshaded coffee				1	1	2								
γ diversity														
Cordillera Central		Colombian Massif				Cordillera Oriental								
5		5				11								

^a Site code describes: municipality (AL = Algeciras, BA = Baraya, GA = Garzón, IQ = Iquira, NE = Neiva, SA = Saladoblanco, Sm = Santa María), site number, and habitat type (fo: forest, tc: traditional coffee, sc: semishaded coffee, and uc: unshaded coffee); ^b Two negative sites were excluded; ^c Five negative sites were excluded.

limited to traditional coffee plantations. Comparison of forest vs. traditional coffee, $\beta = 7$, identified six species unique to forest and one unique to traditional coffee.

UPGMA analyses failed to detect strong clustering by habitat type (Figure 2.3). Nevertheless, five of the eight main groups of sites suggested possible grouping by either forest or by mixed unshaded and semishaded coffee. Most of the sites located in the Cordillera Oriental are grouped into two of the three main branches: 88% of sites in branch 1 (groups 6, 7 and 8) and 75% of sites in branch 2 (groups 3, 4 and 5) are from Cordillera Oriental. The third branch (groups 1 and 2) is dominated by sites from the Cordillera Central and the Colombian Massif.

Based on the dominance of *L. longiflocosa* plus the knowledge that the other relatively common species, *L. nuneztovari*, is not only anthropophilic but has also been incriminated as a CL vector elsewhere, the next sections focus on these two species.

2.3.2 Abundance of the two main sandfly species (*Lutzomyia longiflocosa* and *Lutzomyia nuneztovari*) according to geography

For both species, the numbers collected by outdoor LT were aggregated (Annexes 5 and 6). The index of dispersion (variance/mean ratio) was especially high for *L. longiflocosa*, 1,933 (102,454 / 53) compared with *L. nuneztovari*, 29 (79 / 2.7). The geometric mean (GM) abundance of both sandfly species varied significantly with geography: *L. longiflocosa* (Negative binomial regression, $X^2_{(4)} = 46.36$, $p < 0.0001$) and *L. nuneztovari* (ANOVA, $F_{(6, 447)} = 17.49$, $p < 0.001$). *L. longiflocosa* was most abundant in three municipalities sampled on the Cordillera Oriental (Figure 2.4, Annexe 7): Baraya (GM = 7.1 sandflies/CDC light trap/night, s/LT/n), Algeciras (7.0 s/LT/n), and Neiva (5.9 s/LT/n); and these did not differ significantly in abundance [Baraya vs Algeciras ($z = 0.68$, $p = 0.498$), Baraya vs. Neiva ($z = -1.19$, $p = 0.234$), and Algeciras vs. Neiva ($z = -1.93$, $p = 0.053$)]. Outside the Cordillera Oriental *L. longiflocosa* was found only in Santa María, the more northern of the two sampled municipalities on the Cordillera Central, with a very low abundance, 2.0 s/LT/n. *L. longiflocosa* mean abundance was significantly higher in Baraya compared with the two municipalities where this species was present at low abundance [Santa María ($z = -2.41$, $p = 0.016$) or Garzón ($z = -5.77$, $p < 0.0001$)]. The same geographic pattern in abundance was

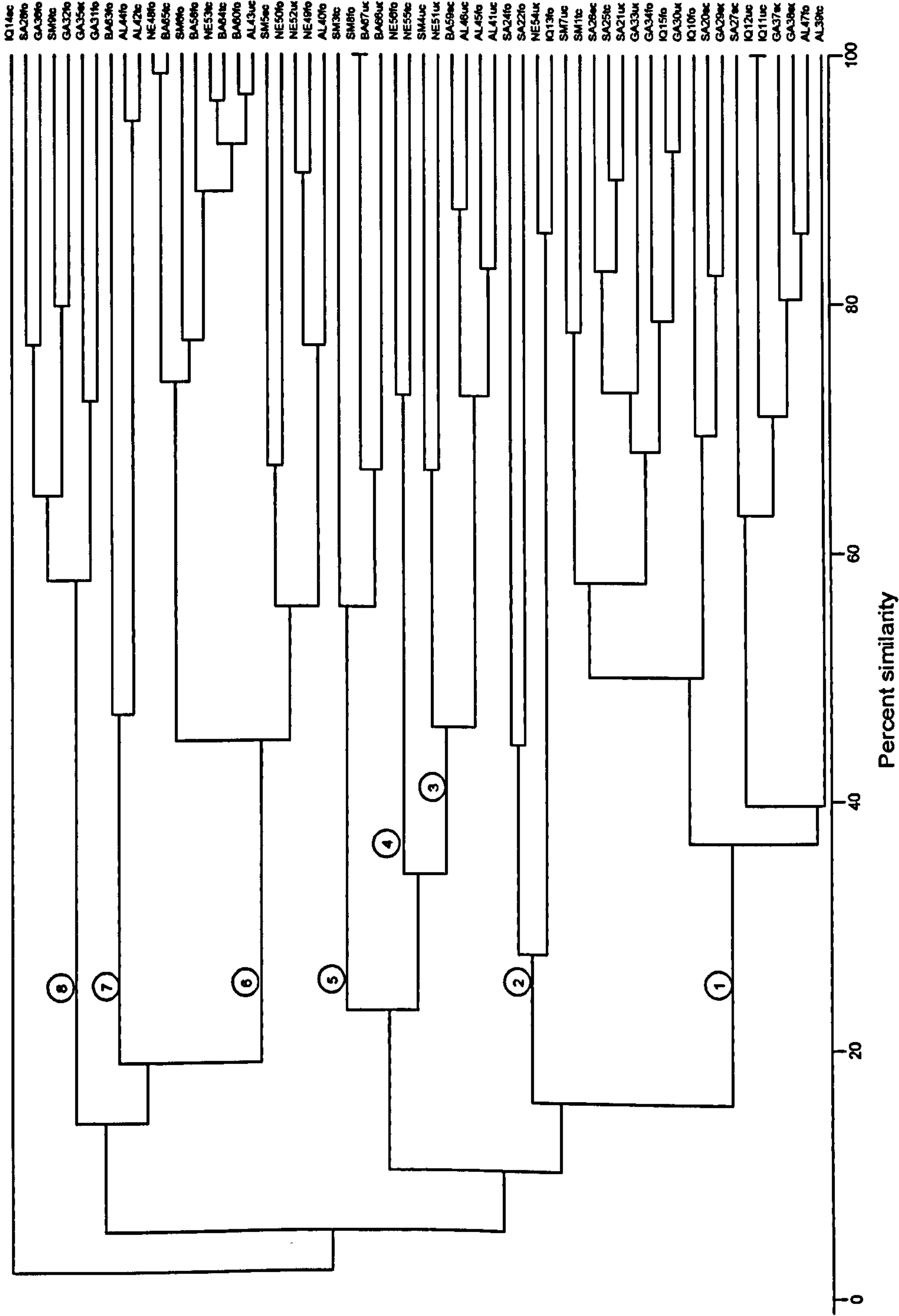


Figure 2.3 Dendrogram based on UPGMA cluster analysis and Percentage Similarity index showing the degree of similarity in anthropophilic sandfly species among 58 sampled sites. Groups of similar sites are marked by numbers enclosed within circles (see text for explanation). On the right hand side of the table there are the site codes (see Table 2.10 for code descriptions).

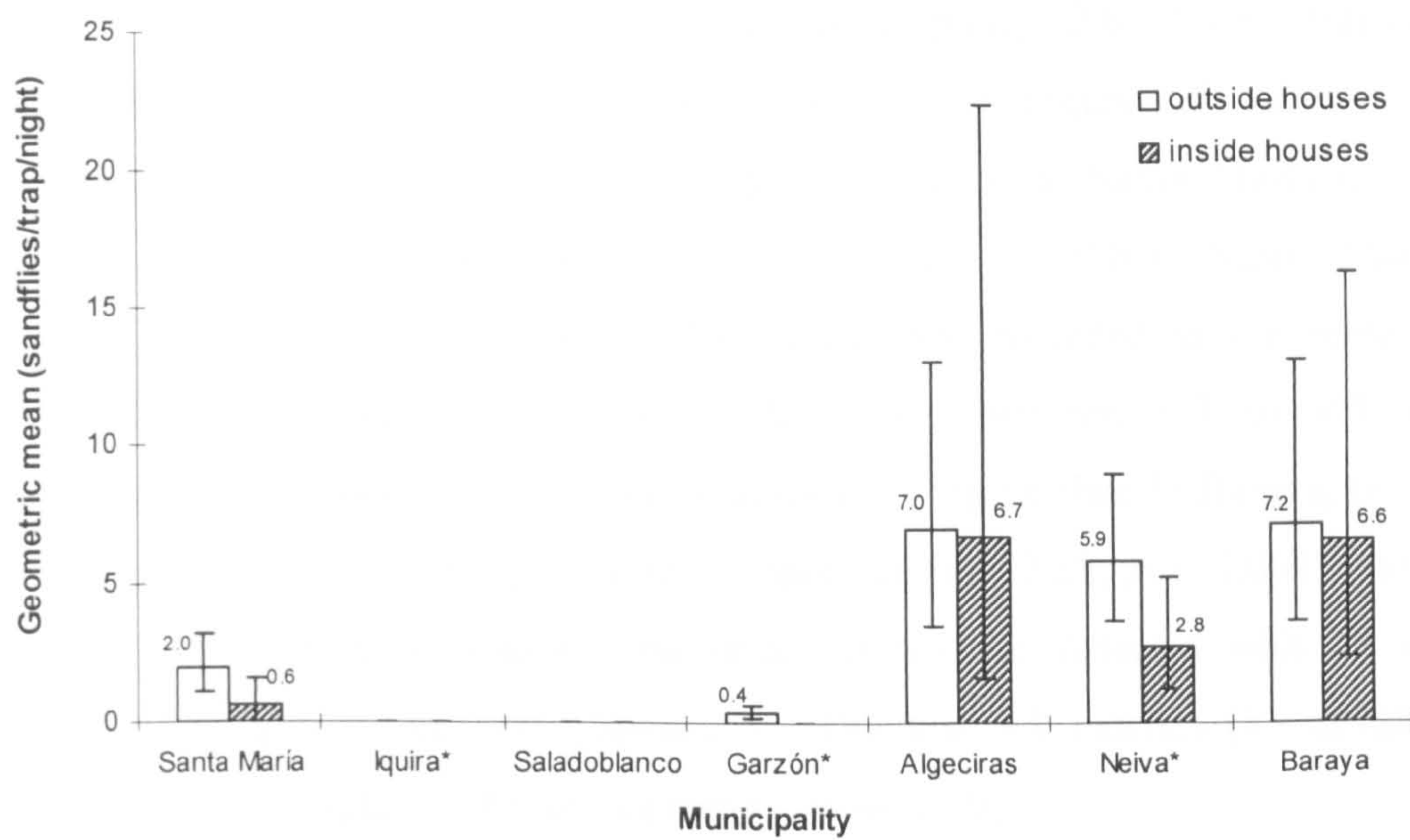


Figure 2.4 The relationship between municipalities and *Lutzomyia longiflocosa* abundance (as measured by CDC light traps). *Some sites were located in neighbouring municipalities. Error bars are the 95% confidence intervals around the geometric means.

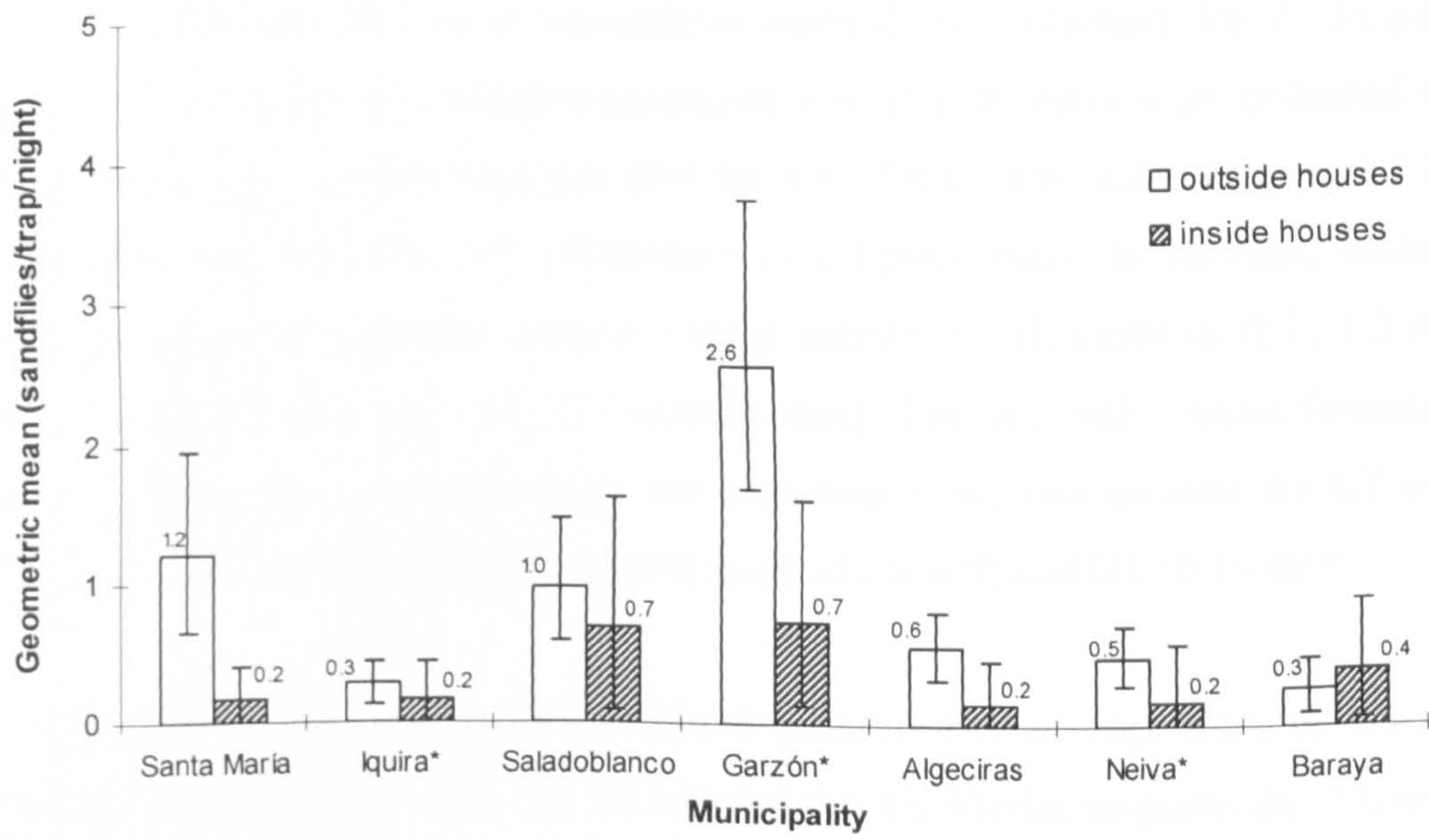


Figure 2.5 The relationship between municipalities and *Lutzomyia nuneztovari* abundance (as measured by CDC light traps). *Some sites were located in neighbouring municipalities. Error bars as in Figure 2.4 .

detected with LT indoors (Figure 2.4, Annexe 8), human landing catches, HL (Annexe 9), and catches by aspiration on tree trunks, AT (Annexe 10).

L. nuneztovari was most abundant in Garzón municipality (2.6 s/LT/n), followed by Santa María (1.2 s/LT/n) and Saladoblanco (1.0 s/LT/n) (Figure 2.5, Annexe 7); and these did not differ significantly in abundance [Garzón vs. Santa María ($z = -1.40$, $p = 0.160$), Garzón vs. Saladoblanco ($z = -1.70$, $p = 0.090$), Santa María vs. Saladoblanco ($z = 0.24$, $p = 0.812$)]. The abundance recorded in the remaining 4 municipalities was relatively constant: GM range between 0.3 to 0.5 s/LT/n. *L. nuneztovari* abundance in Garzón was significantly higher than in Baraya, ($z = -3.76$, $p < 0.001$), Neiva ($z = -3.27$, $p < 0.001$) Algeciras ($z = -2.88$, $p = 0.004$) and Iquira ($z = -3.81$, $p < 0.001$). A similar geographic pattern was detected with LT indoors (Figure 2.5, Annexe 8) and HL (Annexe 9). The few AT catches (13 sandflies) of *L. nuneztovari* prevented any further analyses (Annexe 10).

2.3.3 The effect of location and type of trap on the two main sandfly species catches

2.3.3.1 Trap types

HL was apparently the most productive method of collection for *L. longiflocosa* females. On average, 6.6 females/person/40 min (f/p/40 min) were collected by HL, which is 2.4 times greater than the GM for LT (2.8 f/LT/n) collections and 3.7 times greater than the GM for AT collections (1.8 f/p/40 min). In contrast, males were collected in similar numbers, around 1 male/sample, by all methods (LT, 1.2 m/LT/n; HL, 1.1 m/p/40 min; and AT, 0.7 m/p/40 min). The sex ratio, males/females (m/f), based on the total geometric means for each trap type, was greatest for LT outdoors (0.43 m/f) followed by aspiration on tree trunk (0.39 m/f) and HL (0.18 m/f).

L. nuneztovari females showed a different pattern. The average sizes of LT and HL collections were very similar, 0.6 f/LT/n and 0.5 f/p/40min, respectively. These values were twelve and ten times greater, respectively, than the value obtained by AT (0.05 f/p/40 min) where only three females were collected in the whole project (Annexe 10). Mean abundance of males was four times higher, 0.4 m/LT/n, for LT compared with AT, 0.1 m/p/40 min. No males were collected by HL. The highest sex ratio for this species was found by AT, 2 m/f, followed by outdoor LT, 0.67 m/f. Comparison of

females of the two sandfly species based on HL : LT ratio showed a relatively higher ratio (2.4 : 1) for *L. longiflocosa* than for *L. nuneztovari* (0.83 : 1).

2.3.3.2 Effect of location of CDC light traps

a) Height of the traps in outdoors catches

The following results exclude unshaded coffee (where CDC traps were set up only at 1.5 m height). After controlling by habitat variation, *L. longiflocosa* abundance at 1.5 m above the ground level, GM = 4.6 s/LT/n, was significantly greater than at 10 m, 3.0 s/LT/n ($z = -3.08$, $p = 0.002$) (Table 2.11). This pattern was true for both sexes - females: 4.0 vs. 2.2 f/LT/n; and males: 1.4 vs. 1.2 m/LT/n. The same pattern was observed both in forest, 7.5 vs. 4.4 s/LT/n, and traditional coffee, 5.8 vs. 3.0 s/LT/n. However in semishaded coffee there was no apparent difference in sandfly abundance with trap height (0.5 s/LT/n for each height).

L. nuneztovari mean abundance was slightly greater at 1.5 m, 1.0 s/LT/n, than at 10 m, 0.8 s/LT/n ($z = -2.21$, $p = 0.027$) (Table 2.11), largely due to the effect of height on females (0.8 vs. 0.5 s/LT/n), male abundance being the same at the two heights (0.4 s/LT/n). The effect of height on *L. nuneztovari* was again detected in both forest (1.2 : 1.0 s/LT/n) and traditional coffee (0.8 : 0.4 s/LT/n), but not in semishaded coffee (0.8 s/LT/n for each height).

b) Location of the traps on edge or centre of habitat patch in outdoors catches

L. longiflocosa abundance varied significantly according to trap location in a patch (centre vs edge) depending on habitat type (i.e. there was a significant interaction between habitat and trap position). *L. longiflocosa* abundance was significantly higher on the edge than in the centre of a patch of either forest, 6.5 vs. 5.2 s/LT/n ($z = 5.43$, $p < 0.001$) or traditional coffee, 5.4 vs. 3.3 s/LT/n ($z = -5.43$, $p < 0.001$) (Table 2.12); and this pattern held for both sexes. But the reverse was detected in semishaded coffee, 0.3 vs. 0.7 s/LT/n ($z = 2.4$, $p = 0.017$) and unshaded coffee, 1.3 vs. 2.5 s/LT/n ($z = 2.26$, $p = 0.024$).

Mean abundance of *L. nuneztovari*, unlike for *L. longiflocosa*, after controlling for

Table 2.11 The relationship between habitat type and the height of the CDC light trap.

Habitat	Trap height m	<i>Lutzomyia longiflocosa</i> ^a				<i>Lutzomyia nuneztovari</i>			
		n	GM sandflies/trap/night (95% C.I.)			n	GM sandflies/trap/night (95% C.I.)		
			Female	Male	Total		Female	Male	Total
forest	1.5	75	6.3 (3.6 - 11)	2.1 (1.1 - 3.6)	7.5 (4.2 - 13)	107	0.9 (0.6 - 1.3)	0.5 (0.3 - 0.7)	1.2 (0.8 - 1.7)
	10	76	3.1 (1.8 - 4.9)	1.9 (1.1 - 3.0)	4.4 (2.6 - 7.2)	108	0.6 (0.4 - 0.8)	0.6 (0.3 - 0.9)	1.0 (0.6 - 1.5)
traditional coffee	1.5	40	5.2 (2.1 - 11)	1.9 (0.7 - 3.8)	5.8 (2.3 - 13)	44	0.6 (0.3 - 1.0)	0.3 (0.1 - 0.5)	0.8 (0.4 - 1.3)
	10	40	2.6 (1.2 - 5.0)	1.0 (0.4 - 1.9)	3.0 (1.4 - 5.9)	44	0.3 (0.1 - 0.5)	0.1 (0 - 0.2)	0.4 (0.1 - 0.6)
intensive semishaded coffee	1.5	30	0.4 (0.1 - 0.9)	0.1 (0 - 0.2)	0.5 (0.1 - 0.9)	45	0.6 (0.3 - 0.9)	0.3 (0 - 0.6)	0.8 (0.4 - 1.3)
	10 ^b	26	0.4 (0 - 1.0)	0.2 (0 - 0.5)	0.5 (0.1 - 1.2)	40	0.3 (0.1 - 0.5)	0.4 (0.1 - 0.9)	0.8 (0.3 - 1.4)
total	1.5	145	4.0 (2.6 - 5.9)	1.4 (0.9 - 2.1)	4.6 (3.0 - 6.8)	196	0.8 (0.6 - 1.0)	0.4 (0.3 - 0.5)	1.0 (0.8 - 1.3)
	10	142	2.2 (1.5 - 3.2)	1.2 (0.8 - 1.7)	3.0 (2.0 - 4.2)	192	0.5 (0.3 - 0.6)	0.4 (0.3 - 0.6)	0.8 (0.6 - 1.0)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent; ^b Some samples were taken less than 10 m height.

Table 2.12 The relationship between habitat type and the edge or centre position of the CDC light trap.

Habitat	Trap position	<i>Lutzomyia longiflocosa</i> ^a				<i>Lutzomyia nuneztovari</i>			
		n	GM sandflies/trap/night (95% C.I.)			n	GM sandflies/trap/night (95% C.I.)		
			Female	Male	Total		Female	Male	Total
forest	edge	76	4.9 (2.8 - 8.2)	2.4 (1.3 - 3.9)	6.5 (3.7 - 11)	116	0.7 (0.4 - 0.9)	0.4 (0.2 - 0.6)	0.9 (0.6 - 1.3)
	centre	75	4.0 (2.3 - 6.5)	1.7 (0.9 - 2.7)	5.2 (3.0 - 8.5)	99	0.9 (0.6 - 1.2)	0.7 (0.4 - 1.1)	1.4 (0.9 - 1.9)
traditional coffee	edge	40	4.8 (1.9 - 11)	1.9 (0.7 - 3.9)	5.4 (2.1 - 12)	44	0.3 (0.1 - 0.5)	0.1 (0 - 0.2)	0.4 (0.1 - 0.6)
	centre	40	2.9 (1.3 - 5.7)	1.0 (0.4 - 1.8)	3.3 (1.5 - 6.6)	44	0.6 (0.3 - 1.0)	0.3 (0.1 - 0.5)	0.8 (0.4 - 1.3)
intensive semishaded coffee	edge	28	0.3 (0.1 - 0.6)	0.03 (0 - 0.1)	0.3 (0.1 - 0.6)	41	0.5 (0.2 - 0.8)	0.1 (0 - 0.3)	0.5 (0.3 - 0.9)
	centre	28	0.6 (0.1 - 1.2)	0.3 (0 - 0.6)	0.7 (0.1 - 1.5)	44	0.4 (0.2 - 0.7)	0.6 (0.2 - 1.2)	1.0 (0.5 - 1.8)
intensive unshaded coffee	edge	25	1.2 (0.4 - 2.4)	0.2 (0 - 0.5)	1.3 (0.5 - 2.6)	35	0.3 (0.1 - 0.6)	0.1 (0 - 0.2)	0.4 (0.1 - 0.6)
	centre	25	1.9 (0.5 - 4.7)	1.1 (0.2 - 2.6)	2.5 (0.7 - 6.2)	36	0.3 (0.1 - 0.6)	0.2 (0 - 0.4)	0.4 (0.2 - 0.8)
total	edge	169	3.9 (2.0 - 4.2)	1.3 (0.8 - 1.9)	3.5 (2.4 - 5.1)	236	0.5 (0.4 - 0.6)	0.2 (0.1 - 0.3)	0.7 (0.5 - 0.8)
	centre	168	2.6 (1.8 - 3.6)	1.1 (0.8 - 1.6)	3.2 (2.2 - 4.5)	223	0.6 (0.5 - 0.8)	0.5 (0.3 - 0.7)	1.0 (0.8 - 1.3)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent.

habitat, was significantly higher in the centre, 1.0 s/LT/n, than on the edge, 0.7 s/LT/n, of the habitat patches ($z = 2.59$, $p = 0.009$) (Table 2.12). This was true for both sexes

and in all habitats except unshaded coffee where there was no difference (0.4 s/LT/n for each position).

c) Location indoor vs. outdoor

The ratio of GM females indoor : outdoor catches was greater for *L. longiflocosa*, 0.64 (1.8 / 2.8), than for *L. nuneztovari*, 0.50 (0.3 / 0.6). The same pattern was observed for males. The ratio indoor : outdoor catches of *L. longiflocosa* was especially high in areas of high abundance (Figure 2.4): for instance in Algeciras, 6.7 vs. 7.0 s/LT/n, respectively, and Baraya, 6.6 vs. 7.1 s/LT/n. In contrast, Neiva had a relatively low abundance of sandflies indoor, 2.8 v. 5.9 s/LT/n. In municipalities with low abundance outdoors, the abundance indoors was especially low (Santa María, 0.6 vs. 2.0 s/LT/n) or completely absent (Garzón with only outdoor collections, 0.4 s/LT/n). In contrast, *L. nuneztovari* indoor abundance was relatively independent of outdoor abundance (Figure 2.5). For instance, Garzón and Santa María municipalities with the highest abundance outdoors presented relative low abundance inside houses (0.7 vs. 2.6 s/LT/n for the first and 0.2 vs. 1.2 s/LT/n for the second). In contrast, Baraya, with a low abundance outdoors presented similar abundance inside houses (0.4 vs. 0.3 s/LT/n). For both species, the sex ratio (GM males : GM females) indoors was more female biased than that outdoors, 0.17 (0.3 / 1.8) vs. 0.43 (1.2 / 2.8) m/f, respectively, for *L. longiflocosa*, and 0.2 m/f (0.06 / 0.3) vs. 0.67 (0.4 / 0.6) m/f, respectively, for *L. nuneztovari*.

2.3.3.3 Association between human landing catches (HLC) and CDC light trap catches (LTC) outdoors

For *L. longiflocosa*, outdoor HLC of females was significantly correlated with outdoor LTC (1.5 m above the ground) ($r = 0.87$, $t = 11.55$, $df = 41$, $p < 0.001$) (Figure 2.6). The ln ratio LTC : HLC was 0.8 (1.6 : 2.0), indicating that on average, the total number of sandflies caught on a CDC light trap (18:00 – 7:00 h) was 2.2 times the number of *L. longiflocosa* females collected on a single human bait in 40 min (within 18:00 – 21:00).

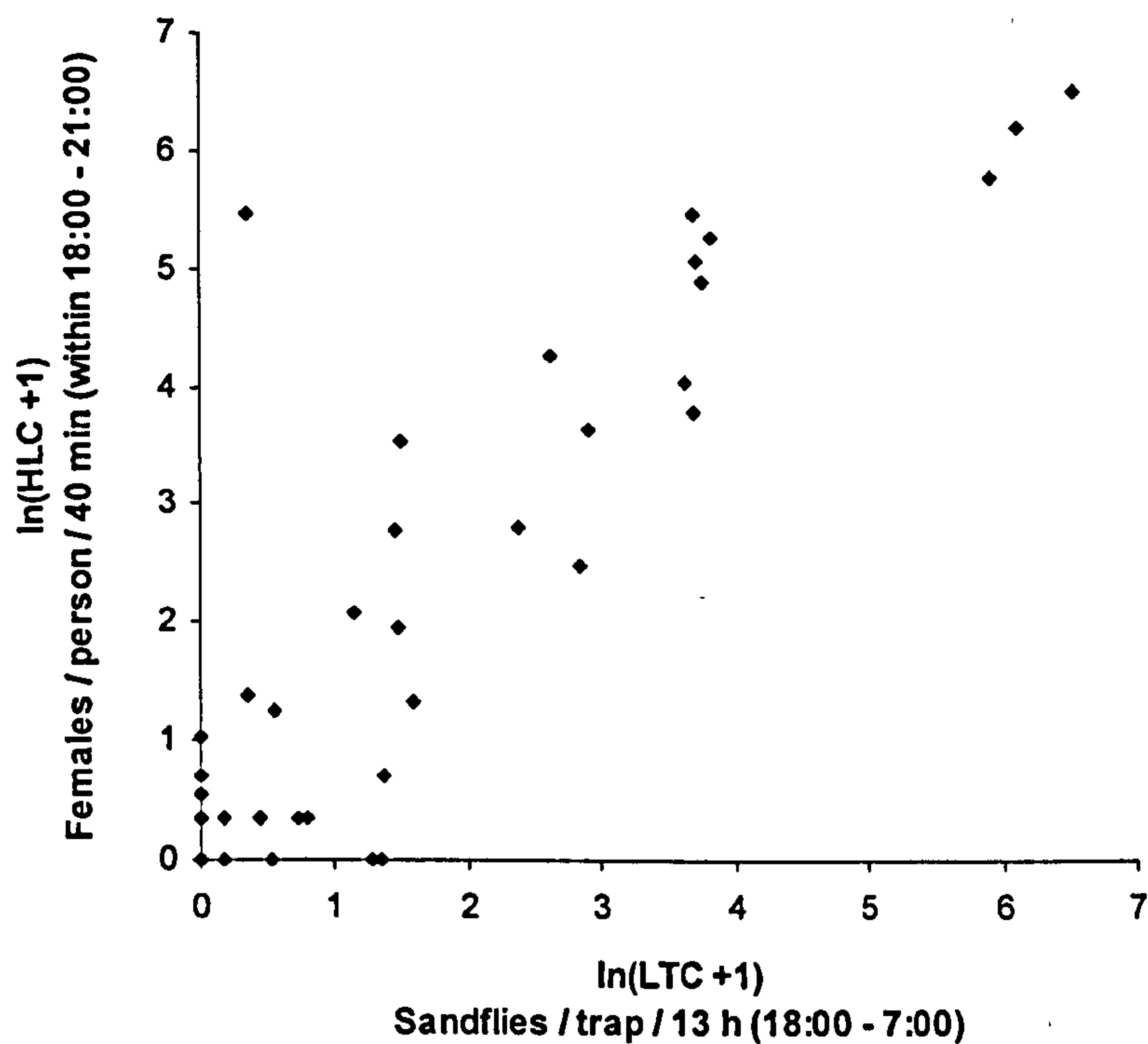


Figure 2.6 The relationship between human landing catches (HLC) of females and CDC, 1.5 m, light traps catches outdoors (LTC) of both sexes for *Lutzomyia longiflocosa*. Each point represents the arithmetic mean of the log transformed data in a sampled site (n = 43).

2.3.4 Local determinants for abundance of the two main sandfly species

2.3.4.1 Association with general habitat types

The sampled mountainous region (940 - 2090 m a.s.l.) belongs to the sub-Andean forest. In this region the primary forest has generally been cleared and replaced with commercial crops, mainly coffee and pasture. As a result the remaining forest is restricted to small patches located along the edge of the riverbanks or at the highest altitude. Coffee plantations are mainly intensive with a few areas covered by traditional coffee growing (19% approximately).

The 67 sampled points were distributed in the following four general habitats: 26 (39%) forests, 11 (16%) traditional coffee plantations, 10 (15%) intensive semishaded coffee plantations, and 20 (30%) intensive unshaded coffee plantations (Table 2.10). A description of these habitats according to the tested local determinants is presented in Table 2.13 . Following there is a description for each habitat:

Table 2.13 Characterization of the general habitats according to the tested local determinants.

Determinant	Forest (No. sites = 26)	Traditional coffee (No. sites = 11)	Intensive semishaded coffee (No. sites = 10)	Intensive unshaded coffee (No. sites = 20)
	Mean or % (95% C.I.)	Mean or % (95% C.I.)	Mean or % (95% C.I.)	Mean or % (95% C.I.)
Protection from wind (% of positive sites)				
protected	31	10	0	0
partially protected	31	45	33	35
unprotected	38	45	67	65
Slope (mean %)	69 (60 - 78)	41 (17 - 64)	29 (13 - 45)	52 (35 - 68)
Distance to the nearest house (m)	227 (137 - 318)	38 (-12 - 89)	6 (-1 - 13)	85 (11 - 160)
Leaf phenology ^a (% of positive sites)				
deciduous	6	55	29	-
semideciduous	44	27	57	-
semideciduous-deciduous	33	18	14	-
semideciduous-evergreen	17	0	0	-
No. of tree strata	2.5 (2.2 - 2.8)	1.6 (1.2 - 2.1)	0.9 (0.5 - 1.3)	0
Cover (mean %)	93 (87 - 100)	84 (72 - 95)	37 (28 - 45)	2 (0 - 5)
Litter cover (mean %)	87 (84 - 90)	76 (68 - 84)	75 (67 - 82)	67 (59 - 76)
Litter depth (cm)				
No decay	3.7 (2.8 - 4.7)	3.8 (0.1 - 7.4)	2.3 (1.0 - 3.6)	3.1 (2.6 - 3.5)
Partial decay	4.7 (3.3 - 6.2)	0.8 (0.4 - 1.3)	0.8 (0.2 - 1.4)	1.0 (0.4 - 1.6)
Total	8.4 (6.9 - 10)	4.6 (1.0 - 8.2)	3.1 (1.4 - 4.8)	4.1 (3.3 - 4.9)
Palms (% of positive sites)	42	0	0	0
Bananas (% of positive sites)	0	82	60	50

^a Eight and three sites with missing values in forest and semishaded coffee, respectively, were excluded; - Variable was not recorded as leaf phenology was recorded only for tree strata which were absent in unshaded coffee.

(a) Forest. Sampling sites in this habitat were located between 940 to 2090 m a.s.l. (Table 2.13). Protected sites were found more frequently (31% of sites) in this habitat than in the coffee plantations. The mean slope was the highest, 69%, compared with the coffee plantations. Semideciduous (44% of sites) and semideciduous-deciduous (33%) forests were the most common according to leaf phenology. Most forests were between

20 to 35 m height, with a mean number of 2.5 tree strata covering 93% of the ground. The mean percentage of litter cover, 87%, and its mean total depth, 8.4 cm, were higher than in any of the coffee planting sites. Some special forms were present according to the altitude. For example, the presence of epiphytes above 1400 m a.s.l. , and palms (exclusive to this habitat) mainly above 1700 m a.s.l. . Forest sites included the largest number of plant species (110) recorded, corresponding to 81% of all the plant species identified within the study. A few species were present in 20% or more of all forest sites: *Quercus humboldtii* (27% of sites), *Cecropia spp.* (23%), *Cyatheaceae spp.* (23%), and *Trophis caucana* (23%).

(b) Traditional coffee plantations. Located between 980 to 1610 m a.s.l., with a mean slope of 41%, most sites were unprotected (45%) or partially protected (45%) from the wind. The most common traditional coffee plantation, according to leaf phenology, was deciduous (56%) followed by semideciduous (27%). Most sites had a similar height, 20 – 35 m, to the forest sites, but with a lower mean number, 1.6, of tree strata, covering 84% of the ground. Litter cover was also lower (76%) than in forest, with a mean total depth of 4.6 cm, around half of that found in forest. In this habitat plant diversity was strongly reduced. Only 24 plant species were identified. Coffee plants were *Coffea arabica* var. típica (91% of sites) which formed a shrub stratum, ≥ 2 m in height, randomly distributed, with a mean density of 1,100 plants/ha . *Musa spp.* (bananas and platano) were very common (82% of sites) in this habitat. Other common plants were *Erythrina sp.* (73%), *Persea americana* (55%), *Cordia alliodora* (36%), *Cassia sp.* (27%), and *Theobroma cacao* (27%). Other fruit trees such as *Manguifera indica* and *Citrus sinensis* were also present. Some species common in forest were also found in traditional coffee plantations: *Inga culagana*, *Inga macrophylla*, *Ficus insipida*, *Cupania americana* and *Bambusa guadua*.

(c) Intensive semishaded coffee plantations. Located between 960 to 1870 m a.s.l., with a mean slope of 29%, these sites were mainly unprotected from wind (67%). The most frequent sites had semideciduous (57%) followed by deciduous (29%) vegetation. The canopy was discontinuous, with high variability in height (median = 10 – 20 m). The mean number (0.9) of tree strata was 2.8 times lower than in forest, covering only 37% of the ground. Litter cover was similar (75%) to that found in traditional coffee, with a mean total depth of 3.1 cm. Nineteen plant species were recorded. Coffee plants were

mostly *Coffea arabica* var. *caturra* (90% of sites), in some cases mixed with *colombia* and *tipica* varieties, forming a shrub stratum, 2 m height. Coffee plants were frequently grown in rows, with a mean density of 5,600 plants/ha. *Musa spp.* (60% of sites) was also very common in this habitat. The most common trees were *Erythrina sp.* (50%), *Mangifera indica* (40%), *Theobroma cacao* (40%), *Citrus sinensis* (30%), *Psidium guajava* (20%), and *Inga spp.* (20%).

(d) Intensive unshaded coffee plantations. Located between 1090 to 1950 m a.s.l., with a mean slope of 52%, these sites were mainly unprotected from wind (65%). This habitat did not have tree strata, although very scarce fruit and forest trees were found in some sites which covered a tiny 2% of the ground. Coffee plants were the dominant species, also mainly *C. arabica* var. *caturra* (90% of sites), sometimes mixed with *colombia* variety. Coffee plants were around 2 m height, distributed in rows with the same plant density, 5,600 plant/ha, found in semishaded coffee. Litter cover was the lowest, 67%, amongst all sampled habitats, with a mean total depth of 4.1 cm. Only 11 plant species were recorded. The second most common plant was *Musa sp.* (50%).

According to outdoor LTC *L. longiflocosa* presented the highest GM abundance in forest, 5.8 (4.0 – 8.3) s/LT/n, followed by traditional coffee, 4.2 (2.4 – 7.2) s/LT/n. The lowest abundance was found in intensive unshaded coffee, 1.9 (0.9 – 3.3) s/LT/n, and intensive semishaded coffee, 0.5 (0.2 – 0.9) f/LT/n (Table 2.14). These differences were significant ($\chi^2_{(3)} = 53.03, p < 0.0001$). *L. longiflocosa* mean abundance in forest was significant greater than that in unshaded coffee ($z = -3.83, p < 0.001$) and semishaded coffee ($z = -6.10, p < 0.001$). No differences were found in *L. longiflocosa* abundance between forest and traditional coffee ($z = -0.61, p = 0.543$). *L. longiflocosa* abundance in traditional coffee plantations was also significantly higher than in unshaded ($z = -3.94, p < 0.001$) or semishaded coffee ($z = -6.04, p < 0.001$). Habitat type was also a significant variable in the multivariate analyses ($\chi^2_{(2)} = 42.59, p < 0.001$), with significant higher abundance in forest than in coffee plantations, except unshaded coffee where the significance was borderline (Table 2.24, Annexe 16).

For *L. nuneztovari* the highest sandfly abundance was also found in forest, 1.1 (0.8 – 1.4) s/LT/n, followed by semishaded coffee, 0.8 (0.5 – 1.2) s/LT/n, and traditional coffee, 0.6 (0.3 – 0.8) s/LT/n. The lowest abundance was found in unshaded coffee, 0.4

Table 2.14 The relationship between general habitat type and outdoor sandfly abundance (as measured by CDC light traps).

Habitat type	<i>Lutzomyia longiflocosa</i> ^a				<i>Lutzomyia nuneztovari</i>			
	GM				GM			
	sandflies/trap/night (95% C.I.)				sandflies/trap/night (95% C.I.)			
	n	Female	Male	Total	n	Female	Male	Total
forest	151	4.4 (3.0 - 6.3)	2.0 (1.3 - 2.8)	5.8 (4.0 - 8.3)	215	0.7 (0.6 - 1)	0.5 (0.4 - 0.7)	1.1 (0.8 - 1.4)
traditional coffee	80	3.7 (2.1 - 6.3)	1.4 (0.7 - 2.3)	4.2 (2.4 - 7.2)	88	0.4 (0.2 - 0.6)	0.2 (0.1 - 0.3)	0.6 (0.3 - 0.8)
intensive semishaded coffee	56	0.4 (0.2 - 0.7)	0.1 (0 - 0.3)	0.5 (0.2 - 0.9)	85	0.4 (0.3 - 0.6)	0.3 (0.1 - 0.6)	0.8 (0.5 - 1.2)
intensive unshaded coffee	50	1.5 (0.7 - 2.7)	0.6 (0.2 - 1.2)	1.9 (0.9 - 3.3)	71	0.3 (0.2 - 0.5)	0.1 (0 - 0.2)	0.4 (0.2 - 0.6)
total	337	2.8 (2.1 - 3.5)	1.2 (0.9 - 1.6)	3.4 (2.6 - 4.3)	459	0.6 (0.4 - 0.7)	0.4 (0.3 - 0.4)	0.8 (0.7 - 1.0)
(total sandflies)		(14346)	(3591)	(17937)		(598)	(636)	(1234)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent.

(0.2 – 0.6) s/LT/n (Table 2.14). These differences were significant ($F_{(3, 453)} = 5.50$, $p = 0.001$), with the abundance in forest significantly higher than that in unshaded coffee ($z = -2.41$, $p = 0.016$). No differences were found when forest was compared with traditional coffee ($z = -1.44$, $p = 0.151$) or semishaded coffee ($z = -0.70$, $p = 0.487$) and between coffee plantation, traditional coffee vs. semishaded coffee ($z = 0.52$, $p = 0.604$), or unshaded coffee ($z = -1.04$, $p = 0.297$), and semishaded vs. unshaded coffee ($z = -1.35$, $p = 0.176$). The multivariate analysis of abundance, where two habitat groups were compared, forest and traditional coffee vs. semishaded and unshaded coffee, also demonstrated a significant association with habitat type ($F_{(1, 390)} = 12.09$, $p = 0.001$), with significant greater abundance in forest and traditional coffee group than that in the group formed by semishaded and intensive coffee (Table 2.25, Annexe 20).

Hence, overall, the ratio of *L. longiflocosa* : *L. nuneztovari* abundance was especially high in the forest, (5.3 : 1), traditional coffee (7 : 1) and unshaded coffee plantations (4.8 : 1). In contrast, the two species had a relative similar abundance in the semishaded coffee plantations (1 : 1.6). The LT collections indoors followed an apparently similar pattern to the outdoor collections for *L. nuneztovari* (Annexe 11). But for *L. longiflocosa* the situation looked different. This species showed the highest indoor abundance in traditional and intensive unshaded coffee plantations, with GM of 4.3 s/LT/n and 3.1 s/LT/n, respectively; followed by forest with 1.7 s/LT/n (Annexe 11).

Finally, HL and AT collections followed a similar pattern to outdoor LT collections for both sandfly species (Annexes 12 and 13), except that (1) no males of *L. nuneztovari* were collected by HL and (2) AT collections of both species were positive only in habitats with well defined tree strata (i.e. forest and traditional coffee sites).

2.3.4.2 Association with specific habitats types, defined by physiognomic-structural classification

Detailed classification of 57 sampled sites according to the physiognomic-structural Kuchler's system, grouped using the TWINSpan software, allowed the conformation of thirteen habitat types (Table 2.15, Annexe 3). Ten forests with poor information on leaf phenology (mainly because difficulties in collecting samples in the field or because samples could not be identified) were excluded from the analysis; and they are presented in Table 2.15 in the category "ungrouped sites". Habitat names were given taking into account the dominant life form, forest height and the number of tree strata. Only life forms with cover $\geq 6\%$ were taken into account in the description.

The majority of habitats, 61% (8 / 13) were only typified by a few sites (1 to 4 sites). The most common habitats (Table 2.15) were: (1) Evergreen low shrub shaded by banana trees (habitat 2, n = 10). The main stratum consists of low (around 2 m tall) coffee plants, shaded, in the majority of cases, by banana trees (2 - 5 m tall). This habitat correspond to unshaded coffee plantations according to the general habitat classification; (2) Evergreen high shrub, sometimes shaded by banana trees (habitat 11, n = 9). The main stratum was high (≥ 2 m tall) coffee plants. Some times there was shade by banana trees (2 – 5 m or 5 – 10 m). This habitat corresponds also to unshaded coffee plantations; (3) Evergreen low shrub shaded by one layer of semideciduous mid trees and banana trees (habitat 3, n = 6). The main stratum was formed by low coffee plants. Shading the coffee there was a layer of semideciduous mid (10 – 20 m tall) trees. Occasionally there was another tree layer (5 - 10 m tall). Finally, there was a layer of banana trees (2 – 5 or 5 - 10 m). This habitat corresponds to a mixture of semishaded coffee and traditional coffee of the general habitats classification; (4) Evergreen high shrub with one layer of deciduous high trees and presence of epiphytes (habitat 13, n = 5). The main stratum was formed by high coffee plants. Shading the coffee there was a layer of deciduous trees (20 – 35 m tall). In this stratum there were epiphytes.

Table 2.15 The relationship between habitat type, according to physiognomic-structural features (Küchler, 1966) grouped using TWINSpan, and sandfly abundance (as measured by CDC light traps and expressed in number of s/LT/n).

Habitat type and description	General habitat description	Küchler formulae (summarizing physiognomic-structural features)	L. longiflora ^a			L. nuneztovari		
			No. sites	n	GM (95% C.I.)	No. sites	n	GM (95% C.I.)
1. Evergreen low shrub shaded by one layer of semideciduous low trees and banana trees. The major stratum consists of evergreen low (0.5 - 2 m tall) shrub (coffee plants), covering ≥ 76% of the ground. Towering there is a layer of semideciduous trees, 5 - 10 m, covering 26 - 50% of the ground. In the middle there is a layer of banana trees, 2 - 5 m tall, covering 6 - 25% or 26 - 50%.	semishaded coffee	B3c S5p H4p	1	20	0.7 (0.2 - 1.4)	1	20	1.9 (0.5 - 4.7)
	semishaded coffee	B3c S5p H4r	1			1		
2. Evergreen low shrub shaded by banana trees. The main stratum consists of evergreen low (0.5 - 2 m tall) shrub (coffee plants), covering ≥ 76% of the ground. Towering the shrub, in the majority of sites, there is a layer of banana trees, 0.5 - 2 m or 2 - 5 m tall, with a variety of covering ranges, 6 - 25% or 26 - 50% or 51 - 75%.	unshaded coffee	B3c5b4b H3r4b	1			1		
	unshaded coffee	B3c H4r	2			3		
	unshaded coffee	B3c H4b	1	32	1.2 (0.3 - 2.7)	1	34	0.3 (0.1 - 0.6)
	unshaded coffee	B3c	2			2		
	unshaded coffee	B3c H4p	2			2		
	unshaded coffee	B3c H4i	1			1		
3. Evergreen low shrub shaded by one layer of semideciduous mid trees and banana trees. Evergreen low (0.5 - 2 m tall) shrub (coffee plants) stratum, covering ≥ 76% of the area. Towering above the shrub there is a layer of semideciduous trees, 10 - 20 m tall, with a variety of covering ranges, 6 - 25% or 26 - 50% or ≥ 76% of the ground. Occasionally there is another tree layer (5 - 10 m tall) covering, 6 - 25% or 26 - 50%. Finally, there is a layer of banana trees, 2 - 5 m or 5 - 10 m tall, covering, 6 - 25% or 26 - 50%.	semishaded coffee	B3c S6p H4r	1			1		
	semishaded coffee	B3c S6p4r H4p	1			1		
	semishaded coffee	B3c S6p H5p	1	48	4.5 (1.9 - 9.4)	1	50	0.6 (0.4 - 0.9)
	traditional coffee	S6c B3c	1			1		
	traditional coffee	B3c S6p5p H4p	1			1		
	unshaded coffee	B3c S6r5r T6a	0			1		
4. Evergreen low shrub shaded by one to three layers of deciduous high trees and banana trees. The major stratum consists of evergreen low (0.5 - 2 m tall) shrub (coffee plants), covering ≥ 76% of the ground. Above this major stratum there are one to three layers of deciduous trees. The first layer, 20 - 35 m tall, covering 6 - 25% or 26 - 50%. The second layer, 10 - 20 m tall, covering 6 - 25% or 51 - 75% of the ground. The third layer, 35 - 40 m tall, covering 6 - 25%. There is also a layer of banana trees, 2 - 5 m tall, covering 26 - 50% of the area.	semishaded coffee	B3c H4p D7r	1			1		
	traditional coffee	B3c D7p8r H4p	1	24	3.3 (1.0 - 8.2)	1	24	0.5 (0.2 - 0.9)
	traditional coffee	D6i7p B3c H4p	1			1		
5. Evergreen low shrub shaded by two layers of trees. The first, deciduous high trees and the second semideciduous mid trees. The major stratum consist of evergreen low (0.5 - 2 m tall) shrub (coffee plants), covering ≥ 76% of the ground. Above this main stratum there are two tree layers, covering 26 - 50% or 51 - 75% of the ground. The first layer is formed by deciduous trees, 20 - 35 m tall. The second layer is formed by semideciduous trees, 10 - 20 m tall. There is also a banana tree layer, 2 - 5 m tall, covering 26 - 50% or 51 - 75% of the ground.	traditional coffee	D7i S6i B3c H4i	1			1		
	traditional coffee	B3c D7p4p S6p H4p	1	20	1.0 (0.02 - 2.9)	1	20	0.1 (0 - 0.3)
	traditional coffee	B3c D7p5p S6p H4p	1			1		

Table 2.15 Continued.

Habitat type and description	General habitat description	Küchler formulae (summarizing physiognomic-structural features)	L. longiflora ^a			L. nuneztoni ^a		
			No. sites	n	GM (95% C.I.)	No. sites	n	GM (95% C.I.)
6. Semideciduous high forest with three tree layers and presence of climbers. The major stratum is a semideciduous layer, 10 - 20 m tall, covering 51 - 75% or ≥ 76% of the ground. There are other two tree layers with the same leaf phenology. the first layer, 20 - 35 m tall, is above the mayor layer, covering 6 - 25% or 26 - 50% or 51 - 75% of the area. The second layer, 5 - 10 m, covering 26% - 50% or 51 - 75% or ≥ 76% of the ground. There are two shrub layers. The first layer, 2 - 5 m tall, covering 26 - 50% or 51 - 75% of the ground. The second layer, 0.5 - 2 m tall, covering 6 - 25% or 26 - 50% or 51 - 75% of the ground. There is high abundance of climber plants in the highest tree stratum.	forest	S6c5c7C***Z ¹ 74i3i T6b	1	24	0.1 (0 - 0.2)	1	24	0.2 (0 - 0.5)
	forest	S6C ¹ i5p7r 74p3r V5p4p	1			1		
	forest	S6i5i7C***p 74p3p	1			1		
7. Forest formed by a mixture of deciduous high and semideciduous mid trees with three tree layers and presence of climbers. The major stratum is a deciduous layer, 20 - 35 m tall, covering 6 - 25% or 26 - 50% or 51 - 75% of the ground. In this stratum there is mid abundance of climber plants. Below the major stratum there are two semideciduous tree strata: the first layer, 10 - 20 m tall, covering 26 - 50% or 51 - 75% or ≥ 76% of the ground, with mid or high abundance of climber plants. The second layer, 5 - 10 m tall, with a variety of covering ranges, 6 - 25 or 26 - 50 or 51 - 75% or ≥ 76% of the ground. Below the tree strata there are two layers of shrub. The first layer, 2 - 5 m tall, covering 6 - 25 or 26 - 50 or 51 - 75% of the ground. The second layer, 0.5 - 2 m tall, also with a wide covering ranges, 1 - 5 % or 26 - 50% or 51 - 75% of the ground.	forest	S6Z ¹ c5r 74i3b D7C**p	1			1		
	semishaded coffee	B3i D6p S5p4r	0			1		
	forest	S6C**i5i 73i4p D7X**p T3r	1			1		
	forest	S5i 74p3p D6C***Z ¹ p7r	1	64	18 (10 - 32)	1	71	1.1 (0.6 - 1.7)
	forest	D7X***i8C**Z ¹ p S6X**i5i 74p3p	1			1		
	forest	S5c6C***p 74i3i D7r	1			1		
	forest	S6C**c5i 74p3p D7r	1			1		
	forest	D7C**W**i S6i5i 74i3p	1			1		
8. Forest formed by a mixture of semideciduous high trees and evergreen mid height plants (palms), with three tree layers and presence of climbers, epiphytes and trees with stilt roots. The mayor stratum consists of a semideciduous layer, 20 - 35 m tall, covering 51 - 75 % or ≥ 76% of the area. In this stratum there is climbers with low or mid abundance and epiphytes in low, mid or high abundance. Below the mayor stratum there is a palm stratum, 10 - 20 m tall, covering 6 - 25% or 26 - 50% of the ground. Next, there is a stratum formed by a mixture of semideciduous trees and evergreen plants (palms), 5 - 10 m tall, with a wide range of covering. Finally, there are two shrub strata. The first, 2 - 5 m tall and the second, 0.5 - 2 m tall, covering 6 - 25% or 26 - 50% of the ground. In this forest there is presence of stilt roots.	forest	S7C**X ¹ i5r H4i B3p T5p6r	1			1		
	forest	S7C***X***i6i5X ¹ Z ¹ p 74i3r T6p5p4r	0	8	3.7 (0.9 - 11)	1	36	1.2 (0.5 - 2.1)
	forest	S7C**X ¹ i5i 73p4r D6X ¹ Z ¹ i T4r6b5b	0			1		
	forest	S7C*X***c6Z ¹ i5i 74p3p T6p	0			1		
9. Semideciduous low forest with one tree layer, abundance of epiphytes and presence of climbers and stilt roots. The major stratum is semideciduous, 5 -10 m tall, covering 51 - 75% of the ground. In this stratum epiphytes are present with mid abundance and there is presence of climbers and stilt roots. Below the major layer there is a shrub layer, 2 - 5 m tall, covering ≥ 76% of the ground. The lowest stratum is formed by forbs 0.5 - 2 m tall, covering 26 - 50% of the ground.	forest	S5C*X**Z ¹ i 74c B6b H3p	0			1	8	9.6 (6.6 - 14)
10. Evergreen high shrub shaded by two layer of semideciduous high trees with presence of epiphytes. Evergreen high, 2 - 5 m tall, shrub (coffee plants) stratum, covering 51 - 75% of the ground. Towering the main stratum there are two layers of semideciduous plants: the first, a layer of semideciduous trees, 20 - 35 m tall, covering 26 - 50% of the area. The second layer, 10 - 20 m tall, covering 6 - 25% of the ground. There is also banana trees, 5 - 10 m, with the same covering	traditional coffee	B4i S7X**p6r H5r	1	8	0.3 (0 - 0.8)	1	8	0.5 (-0.1 - 1.4)

Table 2.15 Continued.

Habitat type and description	General habitat description	Küchler formulae (summarizing physiognomic-structural features)	<i>L. longiflora</i> ^a			<i>L. nuneztoari</i>		
			No. sites	n	GM (95% C.I.)	No. sites	n	GM (95% C.I.)
11. Evergreen high shrub, sometimes shaded by banana trees. Evergreen high, 2 - 5 m tall, shrub (coffee plants) layer, covering ≥ 76% of the ground. In some sites there are also banana trees, 2 - 5 m or 5 - 10 m tall, covering 6 - 25% or 26 - 50% of the ground.	unshaded coffee	B4c H5r	1			2		
	unshaded coffee	B4c H5b	0			1		
	unshaded coffee	B4c D5r	0			1		
	unshaded coffee	B4c H4r	0	18	3.6 (1.2 - 8.3)	1	35	0.5 (0.2 - 0.9)
	unshaded coffee	B4c S6b5b	1			1		
	unshaded coffee	B4c	2			2		
	unshaded coffee	B4c H4p	1			1		
12. Deciduous high or mid forest with three tree layers and presence of epiphytes. The major stratum is a deciduous, 20 - 35 m or 10 - 20 m tall, covering 51 - 75% of the ground. There are two additional deciduous tree strata. The first stratum, 10 - 20 m tall, or 5 - 10 m tall, covering 51 - 75% and 26 - 50% of the ground, respectively. The second stratum could be 5 - 10 m tall or towering, 20 - 35 m tall, covering each one 6 - 26% of the ground. There is a high shrub stratum, 2 - 5 m tall (coffee plants in one site), covering 26 - 50 % or ≥ 76% of the ground. In the stratum 10 - 20 m tall there are epiphytes with low to high abundance.	forest	D6C*X**i5p7r 74p3r	1	8	14 (1.7 - 81)	1	16	0.3 (-0.1 - 0.7)
	traditional coffee	D7i6X'i5r B4c	0			1		
13. Evergreen high shrub with one layer of deciduous high trees and presence of epiphytes. The major stratum is evergreen high, 2 - 5 m tall, shrub (coffee plants), covering in most cases ≥ 76% of the ground. Towering this main layer there is a layer of deciduous trees, 20 - 35 m tall, with a wide covering range, 6 - 25% or 26 - 50% or 51 - 75%. In this stratum there are epiphytes with low, mid or high abundance. Occasionally there is a layer of banana trees, 2 - 5 m or 5 - 10 m tall, covering 6 - 25%.	traditional coffee	D7i B4c H5r3r4b	1			1		
	traditional coffee	D7X**W'i6b B4i V7r6r H4r	1			1		
	semishaded coffee	B4c D7X**p5a H5b	0	16	1.1 (0.1 - 2.9)	1	38	0.9 (0.4 - 1.6)
	semishaded coffee	B4c D7X'r5b H5b4b	0			1		
	semishaded coffee	B4c D7X*p	0			1		
Ungrouped sites	forest	B7p S6p 75p3i4r	1	12	13 (6.2 - 28)	1	12	0.4 (0.1 - 0.8)
	forest	D7C*X'i S5i 74i3i6p	1	8	6.6 (1.9 - 19)	1	8	1.7 (0.4 - 4.2)
	forest	S7C**X**i6p 75i3i4p B3i T4r	1	8	1.8 (0.5 - 4.2)	1	8	13 (5.2 - 31)
	forest	S6X'i 75i3i4p D7p	1	8	1.0 (0.1 - 2.7)	1	8	0
	forest	D7C**W'i D6i 75p4r3r	1	3	0.4 (-0.7 - 6.0)	1	3	0
	forest	76C***i5r T4c	1	8	0.4 (-0.1 - 1.0)	1	8	13 (8.0 - 19)
	forest	77i S6X*p 75p4p3r V6p	0			1	8	0
	forest	77C**X**i6p4r3r5b T6r H4i	0			1	8	0.5 (-0.1 - 1.4)
	forest	77C***X**Z**i6i5r3r T6c5i4p3r H4b	0			1	8	0
	forest	77C*X'i6p5r H4p3r T5b	0			1	4	0
	Total		48	337		67	459	

^a Sites from two areas where *L. longiflora* was absent were excluded; ? : Unknown leaf phenology because of plant samples in some strata were not collected or samples could not be identified.

Occasionally there was a layer of banana trees (2 - 5 m or 5 – 10 m tall). This habitat corresponds to a mixture of semishaded coffee and traditional coffee; (5) Forest formed by a mixture of deciduous high and semideciduous mid trees with three tree layers and presence of climbers (habitat 7, n = 8). The major stratum was a layer of deciduous trees (20 - 35 m tall). Below this stratum there were two semideciduous tree strata, 10 – 20 m and 5 – 10 m tall, respectively. Finally, there were two layers of shrub, 2 – 5 m and 0.2 - 2 m tall, respectively. There were climber plants in the two higher tree strata.

a) Sandfly abundance and specific habitat types

The low number of sites for the majority of identified habitats and the inability to classify a significant part, 38% (10 / 26), of the forest sites (ungrouped habitats) precluded any statistical analysis. Therefore, only a description of the habitats possibly associated with sandfly abundance was carried out.

L. longiflocosa presented its highest abundance, 18 s/LT/n and 14 s/LT/n, in two habitats (Table 2.15): habitats 7 (forest formed by a mixture of deciduous high and semideciduous mid trees with three tree layers and presence of climbers) and habitat 12 (deciduous high or mid forest with three tree layers and presence of epiphytes), respectively. In addition, *L. longiflocosa* abundance was also high, 13 s/LT/n, in an ungrouped forest category (Küchler's formula: B7p S6p ?5p3i4r). These habitats have in common that most of them are forests or forestlike, the majority are high (the highest tree strata 20 – 35 m tall), with three tree strata (Figure 2.7). In general low abundance of *L. longiflocosa* (≤ 1 s/LT/n) was found in four habitats: habitat 1 (evergreen low shrub shaded by one layer of semideciduous low trees and banana trees), habitat 5 (evergreen low shrub shaded by two layers of trees, the first, deciduous high trees and the second semideciduous mid trees), habitat 6 (semideciduous high forest with three tree layers and presence of climbers), and habitat 10 (evergreen high shrub shaded by two layers of semideciduous high trees with presence of epiphytes). Besides the fact that the majority of all these habitats were coffee plantations (semishaded or traditional) no apparent common features were observed.

L. nuneztovari had its highest abundance, 9.6 s/LT/n, in habitat 9 (semideciduous low forest with one tree layer, abundance of epiphytes and presence of climbers and stilt

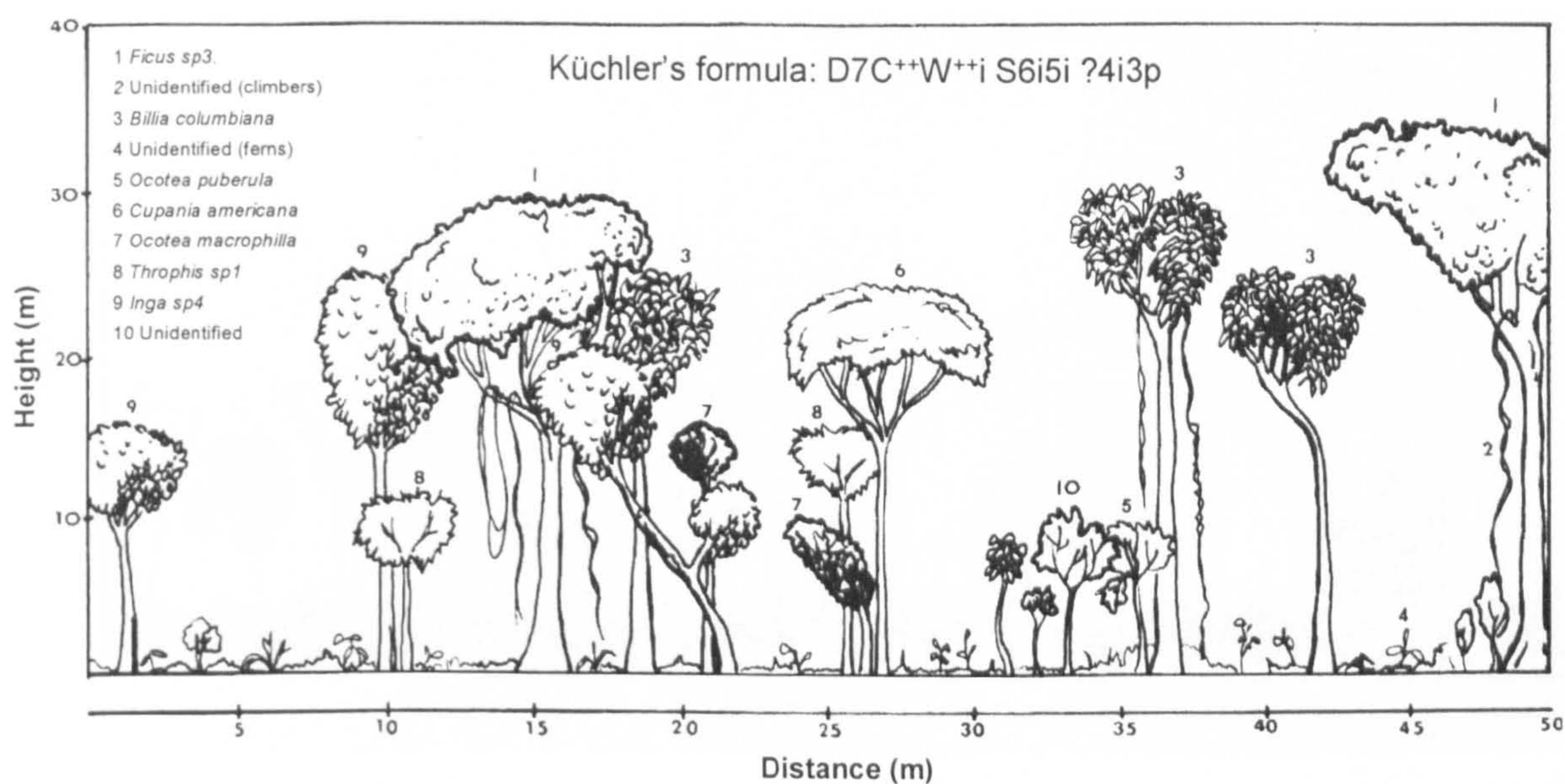


Figure 2.7 Site in La Troja village (Baraya municipality), classified as forest (20 – 35 m tall) formed by a mixture of deciduous high and semideciduous mid trees with three tree layers and with presence of climbers (habitat 7). This habitat presented the highest abundance of *L. longiflocosa*. Drawing by Rocio Cárdenas.

roots); and in two ungrouped forest classes, each one with 13 s/LT/n (Küchler's formulae: S7C++X++i6p ?5i3i4p B3i T4r, and ?6C++i5r T4c, respectively) (Table 2.15). These forests did not have common features, except for the presence of palms trees, in two of the three sites, and climber plants (Figure 2.8). Here forest height were widely variable (from 5 to 35 m tall), as it was the number of tree strata (from 1 to 3). *L. nuneztovari* presented its lower abundance (≤ 0.5 s/LT/n) in seven habitats: the same four described for *L. longiflocosa*, except habitat 1, and habitats 2 (evergreen low shrub shaded by banana trees), habitat 4 (evergreen low shrub shaded by one to three layers of deciduous high trees and banana trees, Figure 2.9), habitat 11 (evergreen high shrub sometimes shaded by banana trees), and habitat 12 (deciduous high or mid forest with three tree layers and presence of epiphytes). No common features for these habitats were observed.

b) Sandfly abundance and individual physiognomic-structural features

Despite difficulties in finding associations between sandfly abundance and specific habitat types, it was apparent that some individual physiognomic structural features (e.g. strata number) could be associated with sandfly abundance. Therefore, some of these

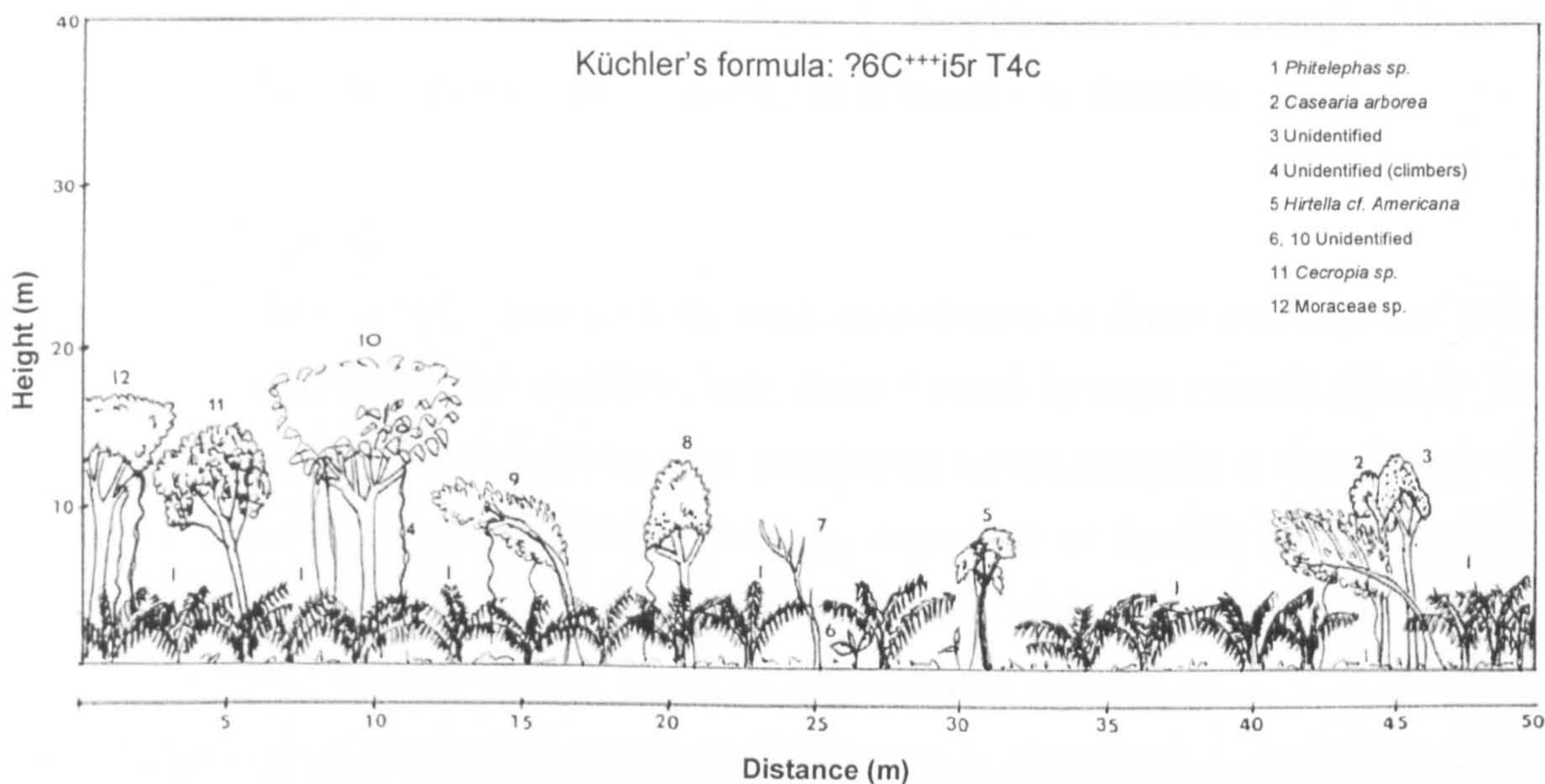


Figure 2.8 Site in Los Guaduales village (Garzón municipality) classified as ungrouped site. This is a forest (10 – 20 m tall) formed by two tree strata and a remarkable palm stratum (*Phitelephas* sp.), with abundance of climbers. This was one of the habitats with the highest abundance of *L. nuneztovari*. Drawing by Rocio Cárdenas.

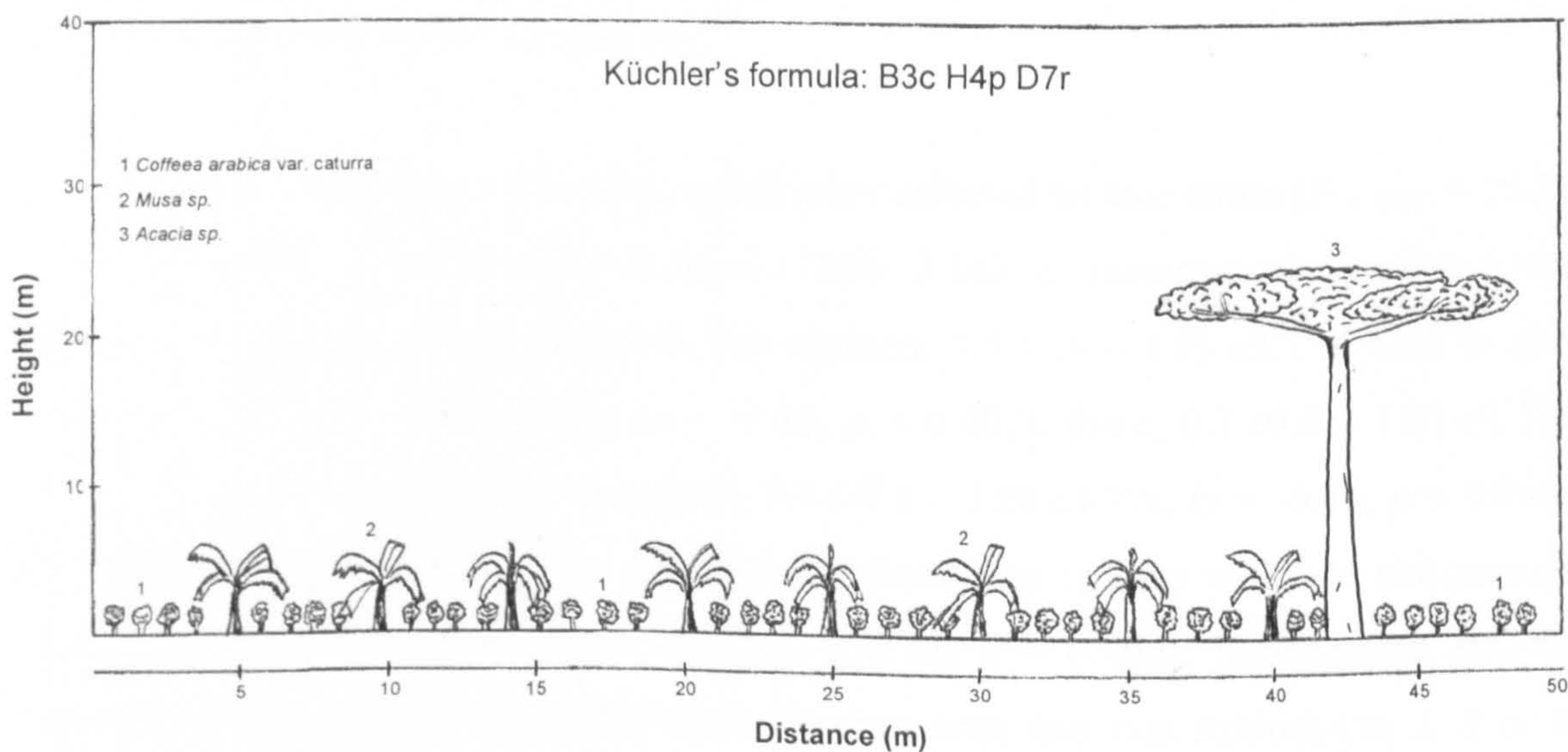


Figure 2.9 Site in Los Guaduales village (Garzón municipality) classified as evergreen low shrub shaded by two three layers of deciduous high trees and banana trees (habitat 4; general classification = semishaded coffee). This habitat presented a relatively low abundance of *L. longiflocosa* and *L. nuneztovari*. Drawing by Rocio Cárdenas.

features (leaf phenology, cover, and number of tree strata) were tested in the analyses. The analyses were carried out on all 67 sampled sites for *L. nuneztovari* and on 48 sites

for *L. longiflocosa* (excluding sites where *L. longiflocosa* was absent). The only significant variables were number of strata and % cover – as described below.

Number of tree strata

Taking into account that trees give the main contribution to forest structure and forest seems the main habitat for sandflies, only strata formed by trees (woody plants ≥ 5 m tall, including palms and bamboos) were considered for the analysis. A tree stratum was defined if trees of a specific height category, regardless of the life forms categories, covered more than 25% of the ground. Mean number of tree strata was 1.4 (Min = 0, Max = 4). The analysis treated strata as a categorical variable (0, 1, 2, 3, 4, strata). Univariate analysis detected significant differences in abundance *L. longiflocosa* with tree strata ($X^2_{(4)} = 130$, $p < 0.001$) (Table 2.16). Specifically, *L. longiflocosa* mean abundance was significantly higher in sites where four tree strata were present, 11 (2.3 - 44) s/LT/n, than in sites with no strata, 1.6 (0.8 - 2.9) s/LT/n, ($z = -7.12$, $p < 0.001$). Multivariate analyses also detected statistical differences in the number of tree strata ($X^2_{(4)} = 69.63$, $p < 0.001$), with *L. longiflocosa* mean abundance greater in sites with four tree strata than in any of the other categories of strata number, including sites with no tree strata (Table 2.24, Annexe 16).

L. nuneztovari abundance was also significantly affected by tree strata ($F_{(4, 449)} = 16.26$, $p < 0.001$), but with the opposite trend (Table 2.16). *L. nuneztovari* abundance was significantly higher in sites with one tree stratum, 1.4 (1.0 - 1.9) s/LT/n, than in sites with two, 0.7 (0.5 - 1.0) s/LT/n, ($z = -3.86$, $p < 0.001$), three, 0.7 (0.4 - 1.0) s/LT/n, ($z = -3.52$, $p < 0.001$) or four tree strata, 0.4 (-0.1 - 1.0) s/LT/n, ($z = -5.15$, $p < 0.001$). No differences were found in *L. nuneztovari* abundance in sites with one tree stratum compared with sites without tree strata ($z = -0.68$, $p = 0.495$). Multivariate analysis confirmed the relatively high abundance in sites with one tree stratum (vs 2, 3 or 4) ($F_{(4, 390)} = 7.53$, $p < 0.001$).

Cover

Cover ranged from 0 to 100%, with a median of 63% ($q_{25} = 3$, $q_{75} = 100$). This variable was treated as continuous for the statistical analysis. Mean abundance of *L. longiflocosa* was apparently higher (> 5 s/LT/n) when cover was high ($\geq 60\%$) and relatively low (≤ 0.6 s/LT/n) when cover was low ($< 60\%$), except for sites with very low

Table 2.16 The relationship between number of tree strata (woody plants, > 5 m tall, in a height category with cover > 25%) and sandfly abundance.

Strata number	<i>Lutzomyia longiflocosa</i> ^a			<i>Lutzomyia nuneztovari</i>		
	n	GM	(95% C.I.)	n	GM	(95% C.I.)
0	54	1.6	(0.8 - 2.9)	83	0.4	(0.3 - 0.6)
1	104	2.0	(1.2 - 3.2)	126	1.4	(1.0 - 1.9)
2	84	5.6	(3.2 - 9.3)	123	0.7	(0.5 - 1.0)
3	87	4.7	(2.8 - 7.5)	119	0.7	(0.4 - 1.0)
4	8	11	(2.3 - 44)	8	0.4	(-0.1 - 1.0)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent.

cover (0 – 19%) where sandfly abundance was relatively high (1.6 s/LT/n) (Table 2.17, where cover is treated as categorical variable). Although univariate analysis did not show any association, multivariate analysis detected a significant positive association between sandfly abundance and cover ($X^2_{(1)} = 39.53, p < 0.001$).

Although *L. nuneztovari* abundance was apparently higher (≥ 1.4 s/LT/n) in sites with relative high cover (40 – 59% and 60 - 79%) compared with the abundance (≤ 0.51 s/LT/n) in sites with low cover (0 – 19% and 20 - 39%), no significant associations were detected with any of the analyses.

2.3.4.3 Association with flora

In total, 183 plant samples were taken for identification and 135 plant species (within 91 genera and 53 families) were identified (Annexe 14). Because of the high diversity in plant species between the 67 sampled sites, it was not possible (with the exception of *Quercus humboldtii*, the only species found of the Fagaceae family) to seek associations between particular plant species and sandfly abundance. Only three plant species, *Q. humboldtii*, *Erithrina* sp. and *Persea americana*, and thirteen families (24% of all families) were common to six or more sites. Furthermore, a potential bias was detected

Table 2.17 The effect of percentage of cover by trees on sandfly abundance.

Cover (%)	<i>Lutzomyia longiflocosa</i>			<i>Lutzomyia nuneztovari</i>		
	n	GM	(C.I. 95%)	n	GM	(C.I. 95%)
0 - 19	54	1.6	(0.79 - 2.9)	81	0.46	(0.29 - 0.66)
20 -39	40	0.6	(0.23 - 1.2)	49	0.51	(0.27 - 0.79)
40 - 59	20	0.2	(0 - 0.42)	42	1.7	(0.84 - 3.1)
60 - 79	44	5.1	(2.6 - 9.2)	60	1.4	(0.88 - 2.2)
80 - 100	179	5.8	(4.0 - 8.2)	227	0.75	(0.55 - 0.98)

at species level as most plant species had aggregated geographical distributions, i.e. found only in municipalities with either high or low abundance of *L. longiflocosa*. At family level the geographic bias were less important. Hence, few generalisations can be made and the analysis was carried out at family level. Ten plant families were common among the whole region: Lauraceae, Myrtaceae, Rubiaceae, Clusiaceae, Moraceae, Melastomataceae, Papilionaceae, Arecaceae, Musaceae and Cyatheaceae. Twenty three families were found only in Cordillera Oriental, two families were unique to the Cordillera Central (Burseraceae and Tiliaceae) and two to the Colombian massif (Chloranthaceae and Symplocaceae). The families Euphorbiaceae, Flacourtiaceae, Mimosaceae and Fagaceae (*Q. hunboldtii*) were present in both the Cordillera Oriental and in the Colombian massif (Annexe 14). The most common families (presence in ≥ 6 sites) were tested as binary variables (presence/absence) by univariate analysis for associations with mean abundance of *L. longiflocosa* and *L. nuneztovari* (Annexe 15). A description of significant associations follows:

a) Plant families positively associated with sandflies

Moraceae

Moraceae (mainly *Ficus spp.* and *Trophis spp.*) was found in forest and traditional coffee plantations. *L. longiflocosa* mean abundance was significantly higher when the family Moraceae was present (15 s/LT/n) than when this family was absent (3.3 s/LT/n) ($z = -2.50$, $p = 0.012$). For *L. nuneztovari*, although the same pattern was apparent, no significant statistical association was detected.

Myrtaceae

Myrtaceae (the majority *Myrcia spp.*, *Eugenia spp.* and *Psidium guajava*) was found mainly in forest and traditional coffee plantations. *L. longiflocosa* mean abundance was apparently higher when the family Myrtaceae was present (11 s/LT/n) than when this family was absent (4.9 s/LT/n), but this difference was not significant. In contrast, *L. nuneztovari* mean abundance was significantly higher when Myrtaceae was present (3.3 s/LT/n) than when this family was absent (0.6 s/LT/n) ($z = -2.37, p = 0.018$).

b) Plant families negatively associated with sandfliesArecaceae

The family Arecaceae (palms) was present mainly in forest habitats. *L. longiflocosa* mean abundance was generally lower when palms were present (1.5 s/LT/n) than when palms were absent (8.3 s/LT/n), and this pattern was confirmed by the univariate analysis ($z = 3.67, p < 0.001$). In contrast, the distribution of *L. nuneztovari* was unaffected by the presence or absence of palms (consistently 1.1 s/LT/n).

Cyatheaceae

This family (tree ferns) was present only in forest habitats. *L. longiflocosa* mean abundance was significantly lower when tree ferns were present (2.6 s/LT/n) than when this plants were absent (6.3 s/LT/n) ($z = 4.74, p < 0.001$). *L. nuneztovari* abundance was unaffected.

Musaceae

This family (mainly *Musa spp.*, known as banana trees), was present in coffee plantations habitats. *L. longiflocosa* mean abundance was significantly lower when this family was present (1.4 s/LT/n) than when this plants were absent (9.0 s/LT/n) ($z = 2.75, p = 0.006$). In contrast, the distribution of *L. nuneztovari* was unaffected by the presence or absence of Musaceae (consistently 0.6 s/LT/n).

Papilionaceae

This family, where *Erythrina sp.* (Cambulo or Cachingo) was the overwhelming dominant species, was found mainly in forest and traditional coffee plantations. *L. longiflocosa* mean abundance was significantly lower when Papilionaceae was present (1.3 s/LT/n) than when this family was absent (8.8 s/LT/n) ($z = 2.78, p = 0.005$). No significant difference was detected for *L. nuneztovari*.

Fagaceae

Fagaceae family, where *Quercus humboldtii* (Roble) was the only reported species, was found only in forest habitats. No significant association was detected with *L. longiflocosa*, but *L. nuneztovari* mean abundance was significantly lower when *Q. humboldtii* was present (0.3 s/LT/n) than when this species was absent (1.5 s/LT/n) ($z = 2.60, p = 0.009$).

2.3.4.4 Association with other habitat features

a) Protection from wind

Protection from the wind was tested as a categorical variable (protected, partially protected and unprotected). Most sampled sites (51.5%) were classified as unprotected from the wind, followed by partially protected sites (34.9%) and protected sites (13.6%). *L. longiflocosa* was most abundant in sites protected from wind (13 s/LT/n) and least abundant in partially protected (3.0 s/LT/n) and unprotected sites (2.0 s/LT/n) (Table 2.18). This difference was borderline non-significant in the univariate analysis, ($X^2_{(2)} = 5.64, p = 0.059$). However multivariate analysis found that *L. longiflocosa* abundance was significantly higher in protected sites than that in unprotected sites ($X^2_{(2)} = 23.45, p < 0.001$) (Table 2.24, Annexe 16). No association with *L. nuneztovari* abundance was identified in any analyses (Table 2.18).

b) Slope in each sampling site

Slope in each sampled site was tested as a categorical variable (eight categories). The majority of sampled sites were classified in categories of high slope: 50.1 – 75% slope (38.8% of sites), 75.1 – 100% slope (16.4%), and 25.1 – 50% slope (10.5%). The mean abundance of *L. longiflocosa* varied significantly with slope ($X^2_{(7)} = 51.26, p < 0.001$), with highest abundance, 8.3 s/LT/n, in sites with 75.1 – 100% slope, and lowest abundance in sites with slopes less than 12.1% (0.1 – 1.6 s/LT/n) (Table 2.19). *L. longiflocosa* abundance in sites with 75.1 – 100% slope was significantly greater than that in sites with 0 – 3% ($z = -5.38, p < 0.001$), 3.1 – 7% ($z = -3.22, p = 0.001$), 7.1 – 12% ($z = -2.14, p = 0.033$), 12.1 – 25% ($z = -2.31, p = 0.021$), and > 100% slope ($z = -3.20, p = 0.001$). Multivariate analysis also detected significant differences in *L. longiflocosa* abundance with slope ($X^2_{(7)} = 181.99, p < 0.001$). Sites with 75.1 – 100%

Table 2.18 The effect of degree of protection from the wind in each sampling site on sandfly abundance.

Degree of protection	<i>Lutzomyia longiflocosa</i> ^a			<i>Lutzomyia nuneztovari</i>		
	n	GM	(95% C.I.)	n ^b	GM	(95% C.I.)
Protected	59	13	(7.4 - 23)	79	0.8	(0.6 - 1.1)
Partially protected	122	3.0	(1.8 - 4.8)	154	0.8	(0.6 - 1.1)
Unprotected	156	2.0	(1.4 - 2.7)	219	0.8	(0.6 - 1.1)

^a GM values exclude data from two municipalities where *L. longiflocosa* was absent;

^b Seven samples with missing data were excluded.

Table 2.19 The effect of slope in each sampling site on sandfly abundance.

Slope %	<i>Lutzomyia longiflocosa</i> ^a			<i>Lutzomyia nuneztovari</i>		
	n	GM	(95% C.I.)	n	GM	(95% C.I.)
0 - 3	6	0.2	(-0.2 - 0.9)	14	0.5	(0.1 - 1.1)
3.1 - 7	12	1.6	(0.1 - 4.8)	31	0.9	(0.4 - 1.7)
7.1 - 12	10	0.1	(-0.1 - 0.3)	18	0.1	(0 - 0.2)
12.1 - 25	36	2.1	(0.7 - 4.7)	52	0.4	(0.2 - 0.7)
25.1 - 50	36	4.5	(1.4 - 11)	59	1.5	(0.9 - 2.4)
50.1 - 75	153	3.4	(2.3 - 4.8)	171	1.1	(0.8 - 1.5)
75.1 - 100	60	8.3	(4.6 - 14)	88	0.6	(0.4 - 0.8)
>100	24	1.5	(0.5 - 3.0)	26	0.4	(0.1 - 0.8)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent.

slope had significantly higher abundance than that in the others ranges of slope, except for the extreme ranges (Table 2.24, Annexe 16).

Both univariate ($F_{(7, 446)} = 4.69, p < 0.0001$) and multivariate analyses also found that *L. nuneztovari* abundance varied significantly with slope. In the former, abundance was significantly higher at sites with 25.1 – 50% slope, 1.5 s/LT/n, than that at sites with 7.1 – 12% slope, 0.1 s/LT/n ($z = -1.99, p = 0.046$). In multivariate analysis, *L. nuneztovari*

abundance was significantly higher at sites with 25.1 – 50% slope than that in any of the other slope categories (Table 2.25, Annexe 20).

c) Litter cover

Litter cover in each sampled site was tested as categorical variable (four categories). The majority of sampled sites were classified in categories of high litter cover: 61 – 80% (58.2% of sites) and 81 – 100% (29.9%). No significant associations with mean abundance of *L. longiflocosa* were detected by univariate analyses (Table 2.20), but multivariate analyses found a significant effect ($X^2_{(3)} = 25, p < 0.001$) (Table 2.24, Annexe 16), with highest abundance, 5.3 s/LT/n, in sites with the highest litter cover, 81 – 100%, and lowest abundance, 0.29 s/LT/n, in sites with the lowest litter cover, 20 – 40%. Similarly no significant associations were detected with *L. nuneztovari* abundance in univariate analyses, but multivariate analysis showed that abundance was significantly higher ($F_{(3, 390)} = 2.65, p = 0.048$) in sites with 41 – 60% litter cover than in sites with 20 – 40% litter cover (Table 2.25, Annexe 20).

d) Litter depth

Measure of litter depth was divided into three components: a) litter with no decay, b) litter partially decayed and c) total litter (the sum of the two). The depth of the litter with no decay ranged from 0 to 20 cm, with a median of 3 cm ($q_{25} = 2, q_{75} = 4$). Partially decayed litter depth ranged from 0.2 to 15 cm, with a median of 1 cm ($q_{25} = 0.5, q_{75} = 3.3$). Total litter depth ranged from 0.8 to 21 cm, with a median of 4.5 cm ($q_{25} = 2.5, q_{75} = 8$). These variables were treated as continuous for the statistical analysis. A positive significant association between depth of no decay litter and mean abundance of *L. longiflocosa* was detected by univariate analysis ($z = 2.15, p = 0.032$) (Table 2.21), but the effect was lost once the outlier was excluded ($z = 1.60, p = 0.110$). Multivariate analysis failed to detect any association. Multivariate (but not univariate) analysis also detected a significant positive association between *L. nuneztovari* abundance and depth of partially decay litter ($F_{(3, 390)} = 3.09, p = 0.027$) (Table 2.21, Annexe 20).

Table 2.20 The effect of percentage of litter cover on sandfly abundance.

Litter %	<i>L. longiflocosa</i>			<i>L. nuneztovari</i>		
	n	GM	(95% C.I.)	n	GM	(95% C.I.)
20 - 40	8	0.29	(-0.16 - 1.0)	8	0.09	(-0.11 - 0.34)
41 - 60	28	1.2	(0.31 - 2.6)	34	0.87	(0.22 - 1.9)
61 - 80	206	3.2	(2.3 - 4.5)	262	0.84	(0.65 - 1.0)
81 - 100	95	5.3	(3.2 - 8.5)	155	0.82	(0.57 - 1.1)

Table 2.21 The effect of depth of litter on sandfly abundance.

Litter type	depth (cm)	<i>Lutzomyia longiflocosa</i> ^a		<i>Lutzomyia nuneztovari</i>	
		n	GM (95% C.I.)	n	GM (95% C.I.)
No decay	0 - 1	44	0.4 (0.1 - 0.7)	63	1.4 (0.9 - 2.2)
	1.1 - 3	192	3.7 (2.7 - 5.0)	278	0.8 (0.6 - 1.0)
	3.1 - 5	61	3.4 (1.9 - 5.7)	70	0.2 (0.1 - 0.3)
	5.1 - 7	16	17 (3.7 - 70)	24	0.9 (0.4 - 1.6)
	> 7	24	6.5 (1.3 - 24)	24	2.3 (0.9 - 4.6)
Partial decay ^b	0 - 1	169	3.2 (2.1 - 4.6)	217	0.7 (0.5 - 0.9)
	1.1 - 2	45	1.2 (0.5 - 2.2)	57	0.3 (0.2 - 0.5)
	2.1 - 3	44	2.7 (1.3 - 4.8)	60	0.9 (0.5 - 1.4)
	3.1 - 4	32	3.0 (1.6 - 5.2)	48	2.0 (1.1 - 3.3)
	> 4	47	11 (5.3 - 23)	75	1.1 (0.6 - 1.8)
Total	0 - 1	8	3.1 (0.3 - 12)	8	3.2 (0.3 - 12)
	1.1 - 3	109	1.6 (1.0 - 2.4)	147	0.7 (0.1 - 0.9)
	3.1 - 5	65	5.2 (2.8 - 9.2)	80	0.4 (0.3 - 0.6)
	5.1 - 7	52	1.9 (1.0 - 3.3)	91	1.0 (0.6 - 1.4)
	7.1 - 9	52	8.9 (4.7 - 16)	64	1.0 (0.6 - 1.6)
	> 9	51	4.6 (2.0 - 9.7)	67	1.2 (0.7 - 1.9)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent; ^b Two samples with missing data were excluded.

e) Distance to the nearest house

The distance between the sampled points outdoors and the nearest house ranged between 0 to more than 600 m . Approximately half of the houses were located either in the sampled habitat or 50 m away (median distance = 50 m, $q_{25} = 0$ m, $q_{75} = 150$ m). For the statistical analysis distance to the nearest house was treated as a continuous variable.

Figure 2.10 (where distance to the nearest house is treated as categorical variable), shows that *L. longiflocosa* mean abundance was high, 6.3 and 8.9 s/LT/n, when houses were close to the sampled site, 1 – 99 m and 100 -199 m, respectively, and low, 3.7 and 0.4 s/LT/n, when houses were far, 300 – 399 and ≥ 400 m, respectively. An exception was the houses located inside the sampled sites where the *L. longiflocosa* abundance was low, 1.6 s/LT/n. Univariate analysis, detected a significant negative relationship between distance to the nearest house and the mean abundance of *L. longiflocosa* ($z = -5.11, p < 0.001$), but multivariate analysis failed to confirm this association.

L. nuneztovari showed a similar pattern: a highly significant negative relationship was detected between distance to the nearest house and mean abundance of *L. nuneztovari* ($z = -2.21, p = 0.027$). *L. nuneztovari* mean abundance was high, 1.0 and 1.3 s/LT/n, when houses were close to the sampled site, 1 – 99 m and 100 -199 m, respectively, and low, 0.2 and 0.3 s/LT/n, when houses were far, 300 – 399 and ≥ 400 m, respectively (Figure 2.11). Multivariate analysis confirmed this association ($F_{(1, 390)} = 11.64, p = 0.001$) (Table 2.25, Annexe 20).

2.3.5 Regional determinants for abundance of the two main sandfly species

2.3.5.1 Association with altitude

Sandflies collections were made between 940 to 2090 m a.s.l. , with a median altitude of 1500 m a.s.l. ($q_{25} = 1180, q_{75} = 1760$). Altitude was tested as a continuous variable including altitude squared to detect a possible nonlinear relationship (probably a normal curve, representative of variables which cause an environmental gradient in the distribution of a species). Analysis is presented for outdoor sandfly catches for this and the others regional determinants. Nevertheless, indoors catches are included in the figures for illustrative purposes.

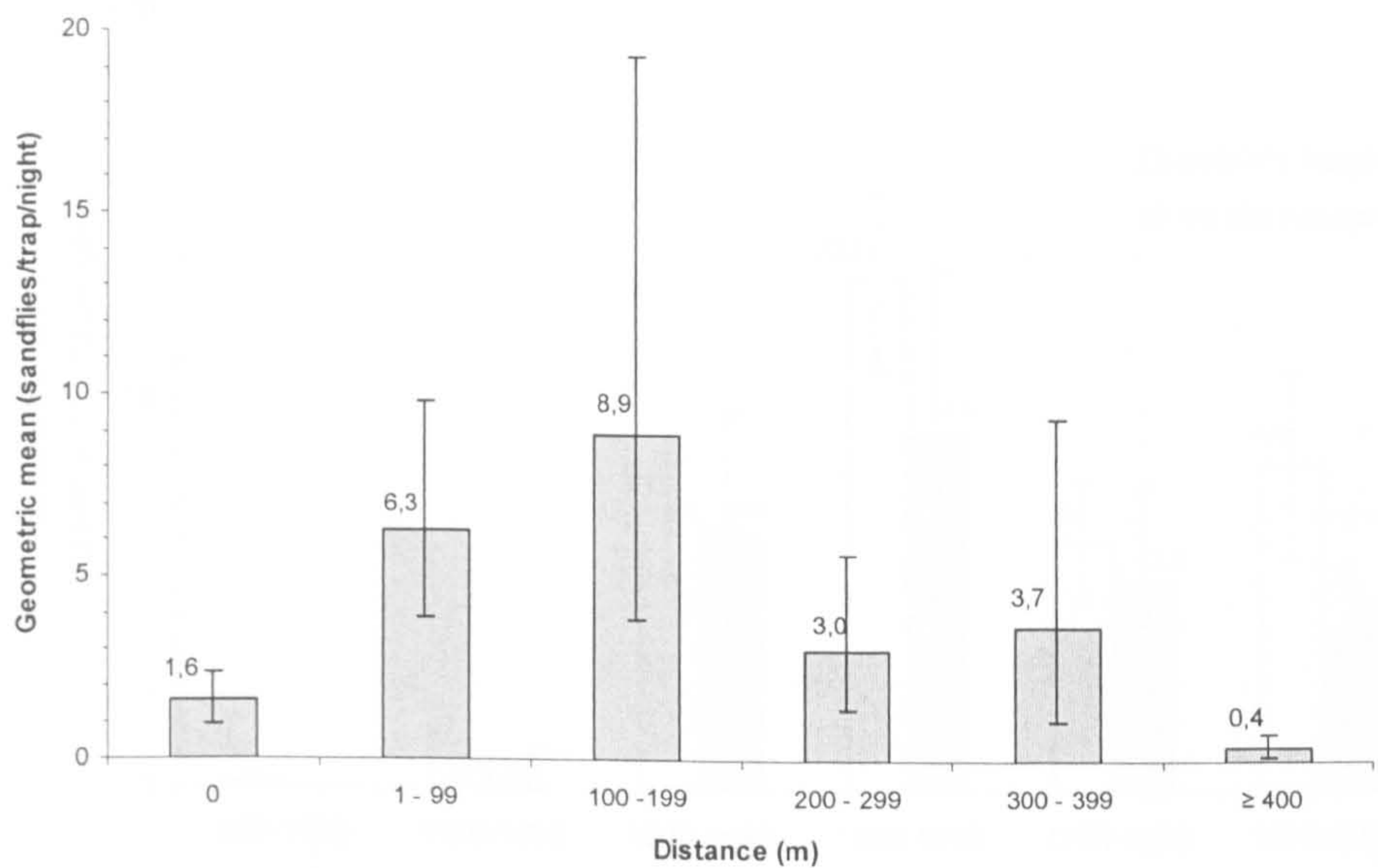


Figure 2.10 The relationship between distance to the nearest house and *Lutzomyia longiflocosa* abundance (as measured by outdoor CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

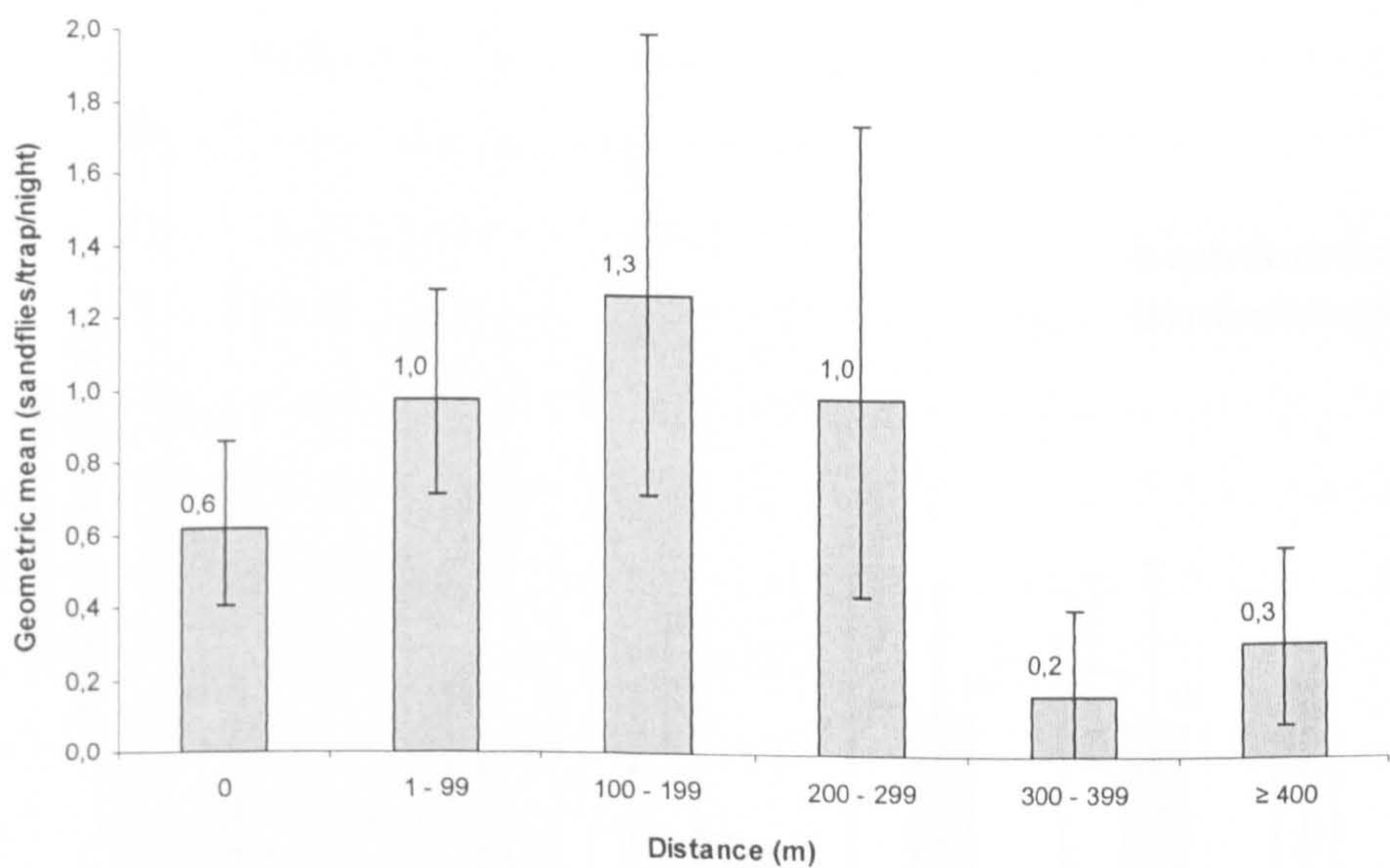


Figure 2.11 The relationship between distance to the nearest house and *Lutzomyia nuneztovari* abundance (as measured by outdoor CDC light traps). Error bars as in Figure 2.10 .

Figures 2.12 and 2.13 show the abundance of *L. longiflocosa* and *L. nuneztovari*, respectively, by altitude category. *L. longiflocosa* was most abundant, GM = 20 s/LT/n, between 1500 to 1699 m a.s.l. . The abundance decreased notably above and below this range: 3.4 s/LT/n and 5.9 s/LT/n in the ranges 1700 – 1899 m a.s.l. and 1900 – 2099 m a.s.l. , respectively, and 0.1 s/LT/n and 0.04 s/LT/n in the ranges 1100 – 1299 m a.s.l. and 900 – 1099 m a.s.l. , respectively. Univariate analyses showed that *L. longiflocosa*

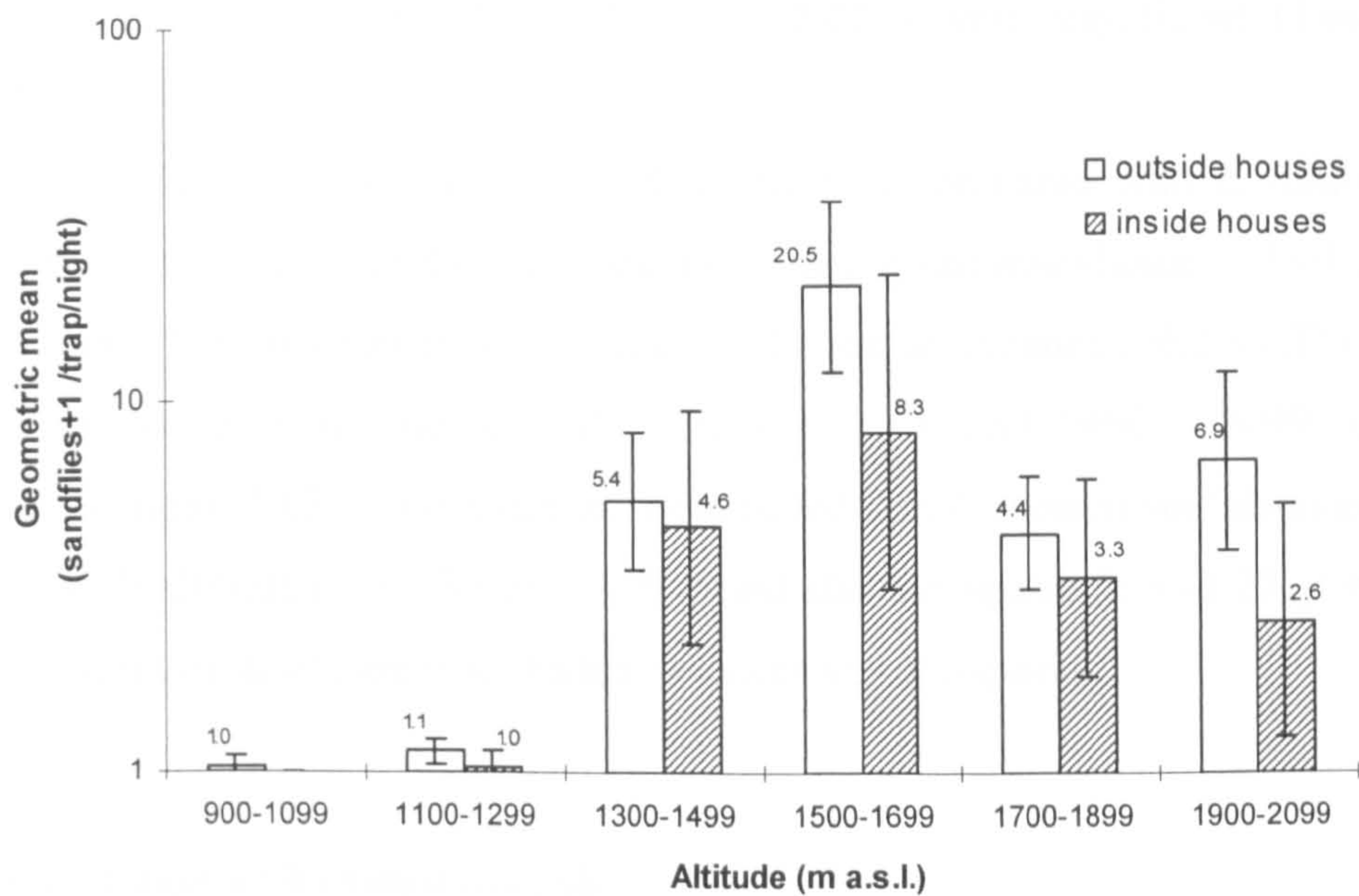


Figure 2.12 The relationship between altitude and *Lutzomyia longiflocosa* abundance (as measured by CDC light traps). The y axis is on a logarithmic scale and the value of 1 was added to make the plot. Error bars are the 95% confidence intervals around the geometric means.

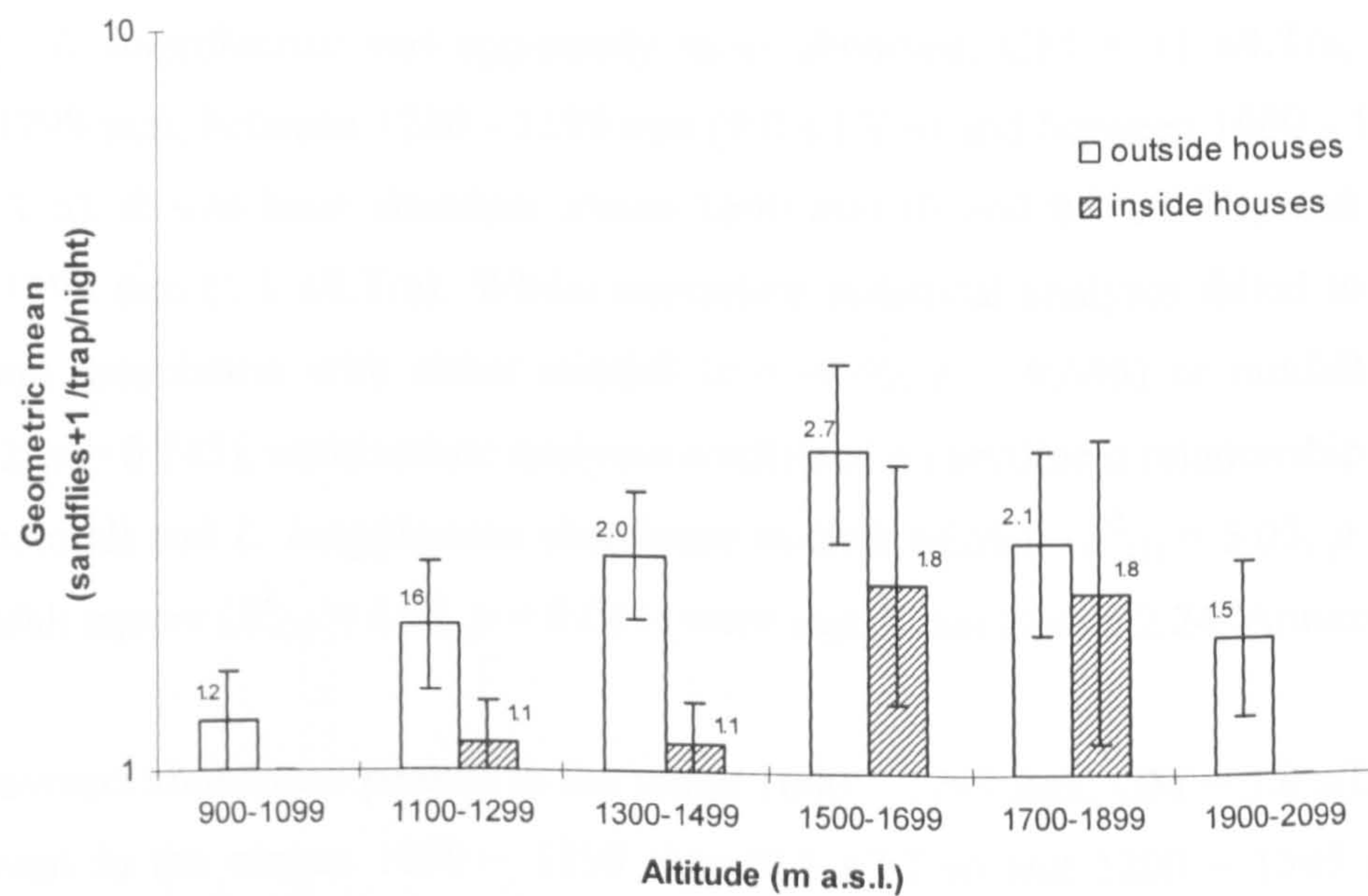


Figure 2.13 The relationship between altitude and *Lutzomyia nuneztovari* abundance (as measured by outdoor CDC light). The y axis and error bars as in Figure 2.12 .

abundance was associated with altitude ($z = 8.56, p < 0.001$) and altitude square ($z = -8.05, p < 0.001$). Multivariate analysis confirmed a curvilinear relationship between *L. longiflocosa* mean abundance and altitude, as both altitude ($X^2_{(1)} = 55.24,$

<0.001) and altitude square ($X^2_{(1)} = 47.03$, $p < 0.001$) were significant (Table 2.24, Annexe 16).

L. nuneztovari showed little variation with altitude as compared with *L. longiflocosa*, except at the extreme ends of the range sampled. Its highest abundance, 1.7 s/LT/n, was again between 1500 to 1699 m a.s.l. ; and the lowest abundances, 0.2 s/LT/n and 0.5 s/LT/n, were found in the ranges 900 - 1099 m a.s.l. and 1900 - 2099 m a.s.l. , respectively (Figure 2.13). Univariate analysis found that *L. nuneztovari* abundance was associated with altitude ($z = 3.36$, $p = 0.001$) and altitude square ($z = -3.27$, $p = 0.001$). Nevertheless, multivariate analyses failed to detect any association.

2.3.5.2 Association with annual rainfall

Mean annual rainfall ranged from 1137 to 2021 mm, with a median of 1562 mm ($q_{25} = 1497$, $q_{75} = 2000$). Rainfall was tested as a continuous variable including rainfall squared to detect a possible nonlinear relationship. Figures 2.14 and 2.15 show the abundance of *L. longiflocosa* and *L. nuneztovari*, respectively, according to rainfall category. *L. longiflocosa* was apparently most abundant, GM = 11 s/LT/n, between 1600 - 1799 mm, between 1200 - 1399 mm (9.0 s/LT/n) and between 1000 - 1199 mm (6.3 s/LT/n). It was least abundant above 1800 mm (0 and 0.9 s/LT/n) and between 1400 - 1599 mm (1.1 s/LT/n). Whilst univariate statistical analyses failed to detect a significant association with either rainfall ($z = -0.40$, $p = 0.686$) or rainfall squared ($z = 0.32$, $p = 0.745$), multivariate analyses confirmed a curvilinear relationship between annual rainfall and *L. longiflocosa* abundance as both rainfall ($X^2_{(1)} = 5.03$, $p = 0.025$) and rainfall square ($X^2_{(1)} = 6.02$, $p = 0.014$) were significant (Table 2.24, Annexe 16).

L. nuneztovari abundance peaked in the range 1600 - 1799 mm, GM = 3.0 s/LT/n, and was lowest in the ranges 1000 - 1199 mm (0.3 s/LT/n) and 1200 - 1399 mm (0.3 s/LT/n). Univariate analyses found a borderline non-significant association with rainfall ($z = 1.89$, $p = 0.059$) but no evidence for curvilinearity ($z = -1.72$, $p = 0.085$); and multivariate analyses confirmed the positive association between rainfall and *L. nuneztovari* abundance ($F_{(1, 390)} = 7.45$, $p = 0.007$) (Table 2.25, Annexe 20).

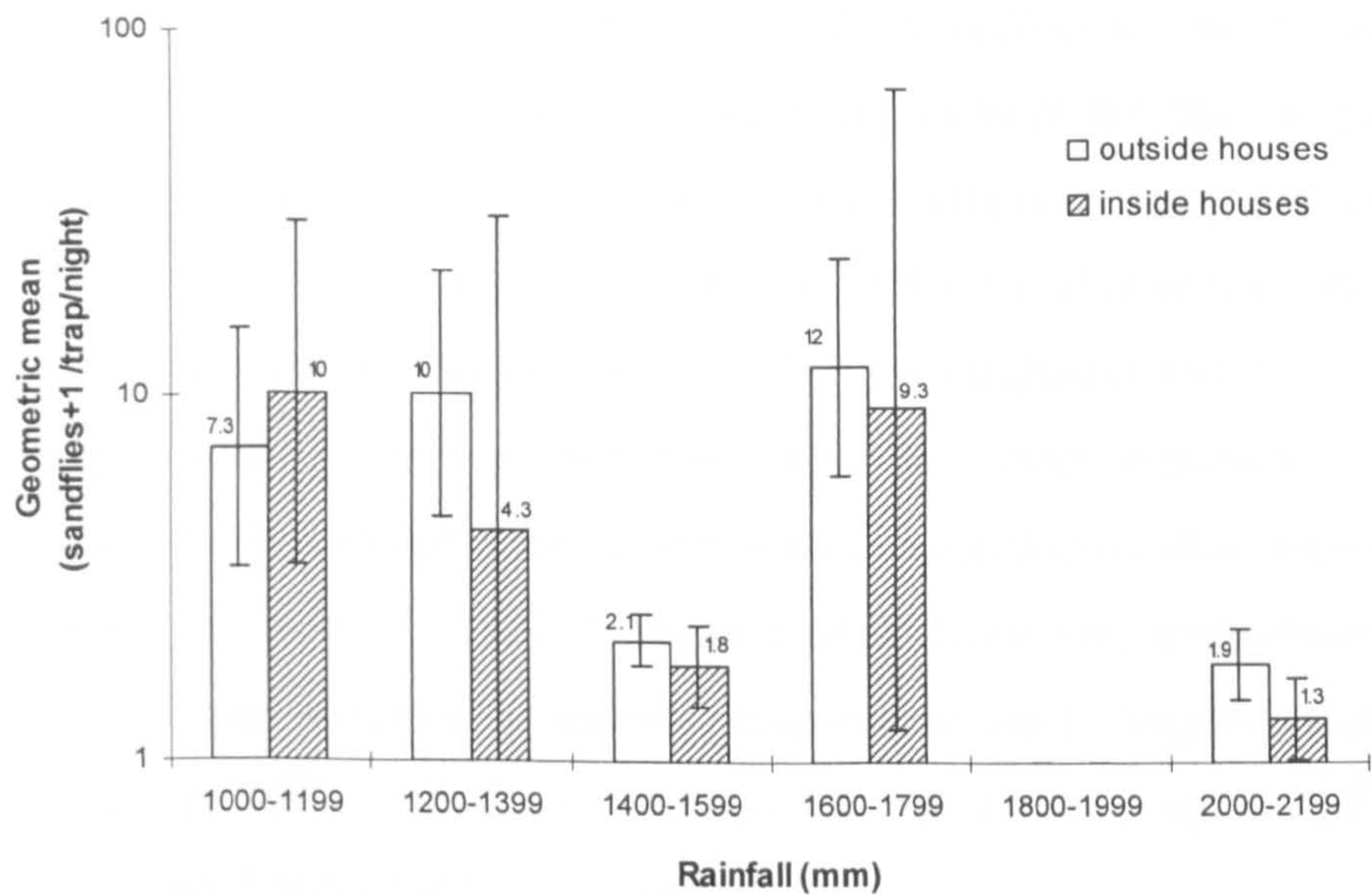


Figure 2.14 The relationship between rainfall and *Lutzomyia longiflocosa* abundance (as measured by CDC light traps). The y axis is on a logarithmic scale and the value of 1 was added to make the plot. Error bars are the 95% confidence intervals around the geometric means. Data included all seven sampled municipalities.

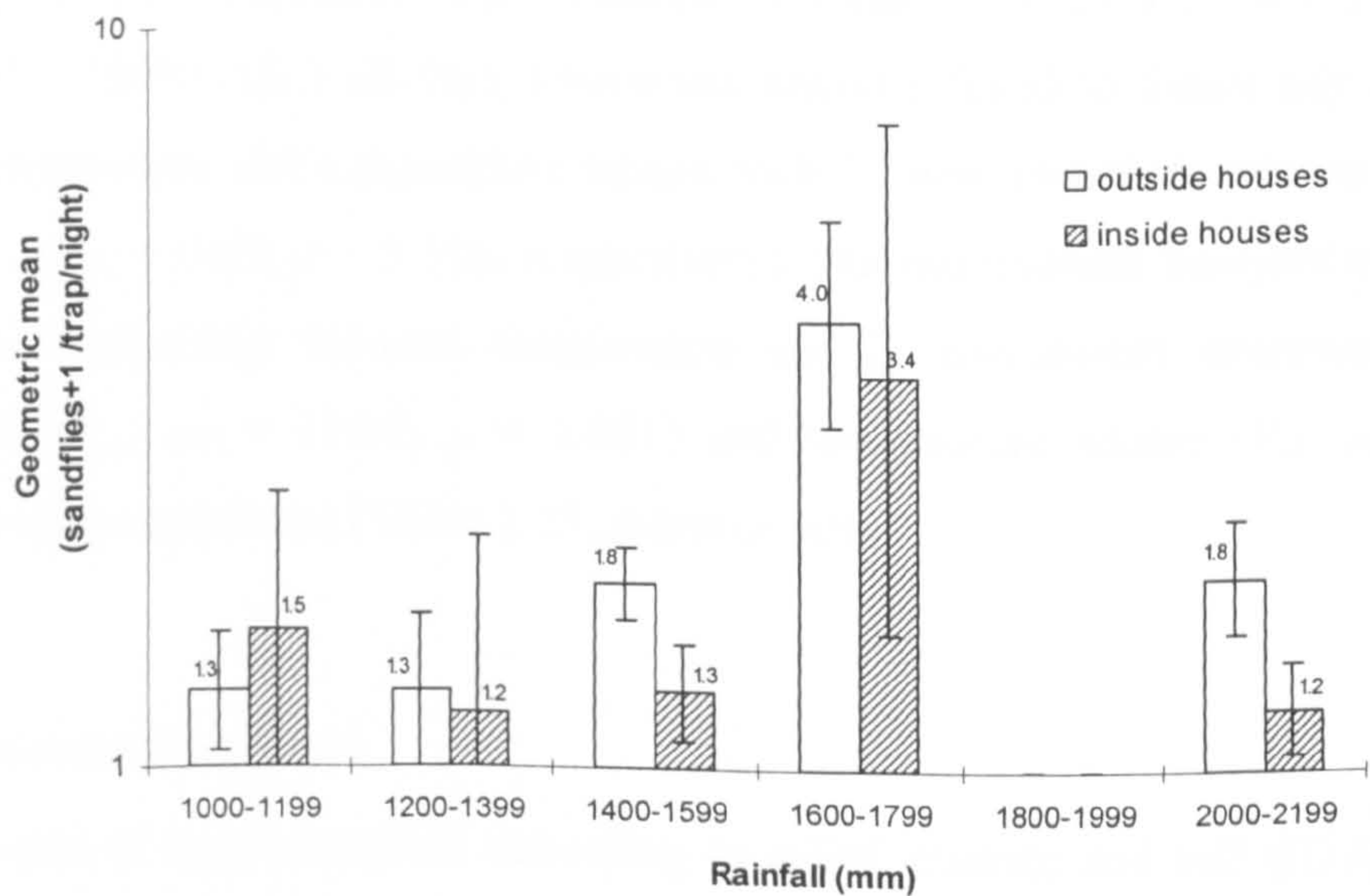


Figure 2.15 The relationship between rainfall and *Lutzomyia nuneztovari* abundance (as measured by CDC light traps). The y axis and error bars as in Figure 2.14 .

2.3.5.3 Association with temperature

Mean temperature ranged from 16 to 23°C, with a median of 19.5°C ($q_{25} = 18.6$, $q_{75} = 21.9$). Temperature was tested as a continuous variable including temperature square to detect a possible nonlinear relationship.

Figures 2.16 and 2.17 show the abundance of *L. longiflocosa* and *L. nuneztovari*, respectively, according to temperature category. Samples were not taken in the range 17 – 17.9°C. *L. longiflocosa* was most abundant in two contiguous ranges of temperature: 18 - 18.9°C (GM = 4.5 s/LT/n) and 19 - 19.9°C (4.3 s/LT/n). This species was absent or rarely present in the extreme ranges, 16 – 16.9°C (no sandflies) and 23 - 23.9°C (0.1 s/LT/n) (Figure 2.16). Univariate analyses failed to detect significant association between temperature and temperature square with *L. longiflocosa* abundance ($z = 1.14$, $p = 0.253$; and $z = -1.18$, $p = 0.237$, respectively). However, multivariate analyses confirmed a curvilinear relationship between temperature and *L. longiflocosa* abundance as both temperature ($X^2_{(1)} = 9.05$, $p = 0.003$) and temperature square ($X^2_{(1)} = 8.86$, $p = 0.003$) were significant (Table 2.24, Annexe 16).

L. nuneztovari was found in all the sampled ranges of temperature. The highest abundance was found between 22 – 22.9°C (GM = 1.9 s/LT/n). A high abundance was also found between 19 – 19.9°C and between 23 – 23.9°C (1.6 s/LT/n each) (Figure 2.17). The lowest abundance was detected between 21 – 21.9°C (0.1 s/LT/n) and between 20 – 20.9°C (0.2 s/LT/n). Univariate analysis failed to detect any association between temperature and temperature square with *L. nuneztovari* abundance ($z = -0.57$, $p = 0.566$; and $z = 0.65$, $p = 0.516$, respectively). But multivariate analyses confirmed a curvilinear relationship between temperature and *L. nuneztovari* abundance as both temperature ($F_{(1, 390)} = 12.67$, $p < 0.001$) and temperature square ($F_{(1, 390)} = 13.78$, $p < 0.001$) were significant (Table 2.25, Annexe 20).

2.3.5.4 Association with soil

Thirteen types of soil, classified according to relief, climate and soil (IGAC Instituto Geográfico Agustín Codazzi 1994), were identified within the study area (Table 2.22). The majority (around 60%) of the 64 sampled sites, where soil was identified, corresponded to four types of mountainous soils: associations Entic Hapludolls - Andic Humitropepts – Lithic Troorthents, MQE, and Oxic dystropepts – Typic Troorthents, MQA, (17.2%, each), which are soils of humid mid climate; association Lithic Ustorthents – Typic Haplustolls, MRA (15.6%), soil of dry mid climate; and the association Typic Humitropepts – Typic Troorthents – Typic Hapludands, MLB (10.9%), soil of humid cold climate.

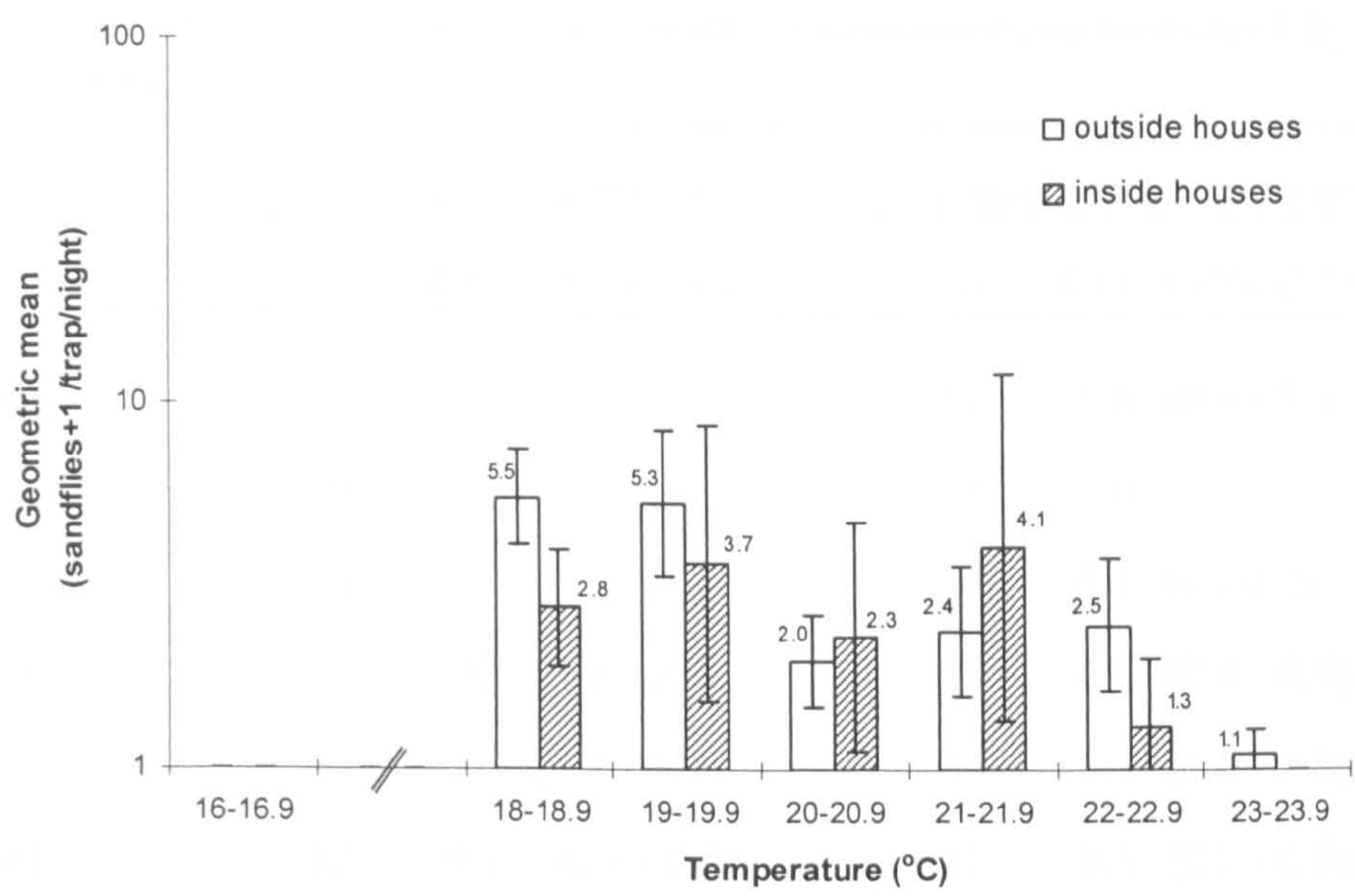


Figure 2.16 The relationship between temperature and *Lutzomyia longiflocosa* abundance (as measured by CDC light traps). The y axis is on a logarithmic scale and the value of 1 was added to make the plot. Error bars are the 95% confidence intervals around the geometric means. Data included all seven sampled municipalities.

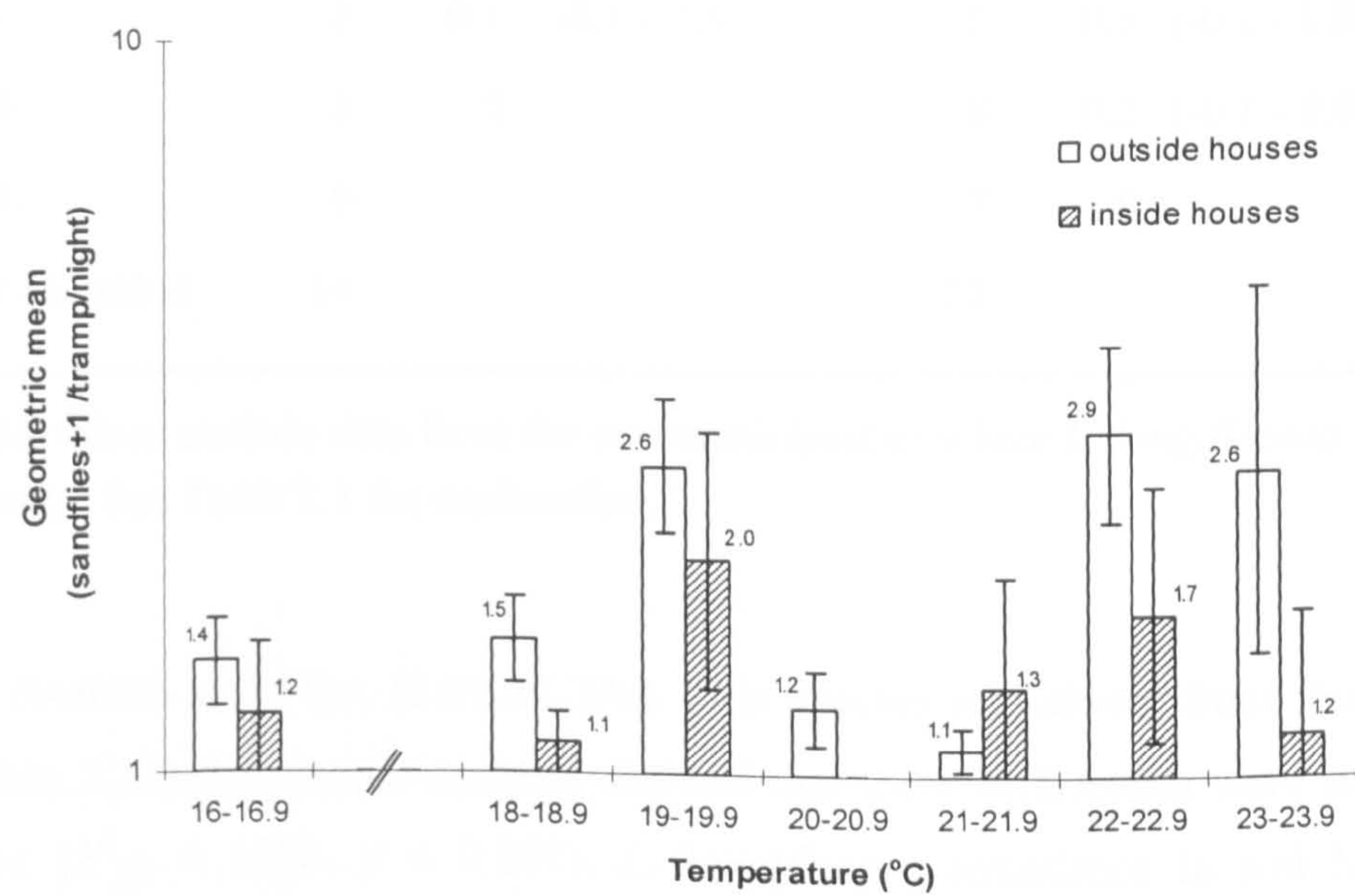


Figure 2.17 The relationship between temperature and *Lutzomyia nuneztovari* abundance (as measured by CDC light traps). The y axis and error bars as in Figure 2.16 .

The highest *L. longiflocosa* abundance was found in mountainous soils of humid mid climate: MQA (13 s/LT/n), MQG (9.1 s/LT/n), MQM (8.3 s/LT/n), and in a mountainous soil of dry mid climate, MRA (4.3 s/LT/n). The lowest *L. longiflocosa* abundance was found in a mountainous soil of humid mid climate MQD (0.06 s/LT/n)

Table 2.22 The effect of type of soil on sandfly abundance (as measured by outdoor CDC light traps).

Soil type ^b	<i>Lutzomyia longiflocosa</i> ^a			<i>Lutzomyia nuneztovari</i>		
	n	GM	(95% C.I.)	n	GM	(95% C.I.)
AQC	0			11	1.6	(0.6 - 3.4)
LXA	0			8	0	
MLB	0			36	0.1	(0 - 0.2)
MQA	51	13	(6.3 - 25)	74	0.6	(0.4 - 0.9)
MQC	45	3.1	(1.7 - 5.4)	45	1.8	(1.0 - 3.0)
MQD	12	0.1	(-0.1 - 0.2)	41	0.5	(0.2 - 0.7)
MQE	87	1.4	(0.9 - 2.2)	87	2.0	(1.3 - 2.9)
MQG	24	9.1	(3.7 - 21)	24	0.3	(0 - 0.7)
MQM	32	8.3	(4.3 - 15)	32	0.9	(0.5 - 1.4)
MRA	56	4.3	(1.9 - 8.9)	56	0.2	(0 - 0.4)
PQA	8	0.1	(-0.1 - 0.3)	8	0.3	(-0.1 - 0.8)
PQF	8	0		8	0.2	(-0.1 - 0.6)
PXG	0			7	0	
Not identified	14			22		

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent; ^b See Table 2.3 for explanation.

and in a foothills soil PQA (0.09 s/LT/n). This species was absent from foothills soil PQF (Table 2.22). Univariate analysis showed that these differences were statistically significant ($X^2_{(8)} = 1858, p < 0.001$). *L. longiflocosa* abundance in soil MQA was significantly greater than that in soils MQC ($z = -2.03, p = 0.043$), MQD ($z = -11.93, p < 0.001$), MQE ($z = -3.16, p = 0.002$), MQM ($z = -2.36, p = 0.018$), PQA ($z = -11.23, p < 0.001$) and PQF ($z = -19.05, p < 0.001$). Nevertheless, no significant differences in *L. longiflocosa* abundance were found between MQA compared with MQG ($z = -0.89, p = 0.371$) and MRA ($z = -0.66, p = 0.508$). Multivariate analysis confirmed the statistical significant difference in *L. longiflocosa* abundance according to soil type

($X^2_{(7)} = 236.51$, $p < 0.001$). *L. longiflocosa* abundance in soil MQA was significantly higher than that in soils MQC, MQD, MQM and MRA (Table 2.24, Annexe 16).

In contrast, the highest *L. nuneztovari* abundance was found in two mountainous soils of humid mid climate, MQE (2.0 s/LT/n), MQC (1.8 s/LT/n), and in one soil, AQC (1.6 s/LT/n), of plateau with the same type of climate. The lowest *L. nuneztovari* abundance was found in a variety of soils: mountainous humid cold climate, MLB (0.07 s/LT/n), humid mid climate, MQG (0.27 s/LT/n), dry mid climate, MRA (0.23 s/LT/n), and foothills humid mid climate, PQF (0.19 s/LT/n) and PQA (0.25 s/LT/n). *L. nuneztovari* was absent from soils of dry and very dry hot climate located on low mountains, LXA or foothills, PXG (Table 2.22). Univariate analysis showed that these differences in soil were statistically significant ($F_{(34, 419)} = 4.18$, $p < 0.0001$). *L. nuneztovari* abundance in soil MQE was significantly greater than that in soils MQG ($z = -2.41$, $p = 0.016$), PQA ($z = -2.90$, $p < 0.004$), PQF ($z = -2.42$, $p = 0.016$), MQD ($z = -2.06$, $p = 0.039$), MRA ($z = -2.39$, $p < 0.017$), MLB ($z = -3.11$, $p = 0.002$), PXG ($z = -2.48$, $p = 0.013$), and LXA ($z = -3.36$, $p < 0.001$). Nevertheless, no significant differences in *L. nuneztovari* abundance were found between MQE compared with MQM ($z = -1.34$, $p = 0.179$), AQC ($z = 0.28$, $p = 0.780$), MQA ($z = -1.62$, $p = 0.106$), and MQC ($z = 0.09$, $p = 0.925$). Multivariate analysis confirmed the statistical significant difference in *L. nuneztovari* abundance according to soil type ($F_{(10, 390)} = 15.33$, $p < 0.001$). *L. nuneztovari* abundance in soil MQE was significantly higher than that in all the other soil categories, except soils PQA and MQC (Table 2.25, Annexe 20).

2.3.5.5 Association with slope of the general relief

Slope of the general relief was tested as a categorical variable (six categories). The majority (around 94%) of 63 sampled sites, where slope could be recorded, were classified in three categories: 50.1 – 75% slope (71.4% of sites), 25.1 – 50% slope (12.7%), and 3.1 – 7% slope (9.5%). *L. longiflocosa* abundance varied significantly with slope in the univariate analyses ($X^2_{(3)} = 29.51$, $p < 0.001$). *L. longiflocosa* abundance in 25.1 – 50% slope was significantly greater than that in the other three categories of slope: 50.1 – 75% ($z = -2.4$, $p < 0.016$), > 75% ($z = -5.35$, $p < 0.001$), and 3.1 – 7% slope ($z = -2.23$, $p = 0.026$) (Table 2.23). Multivariate analysis confirmed this association ($X^2_{(3)} = 122.69$, $p < 0.001$), with *L. longiflocosa* abundance in sites with

Table 2.23 The effect of slope of the general relief on sandfly abundance (as measured by outdoor CDC light traps).

Slope %	<i>Lutzomyia longiflocosa</i> ^a			<i>Lutzomyia nuneztovari</i>		
	n	GM	(95% C.I.)	n	GM	(95% C.I.)
3.1 - 7	40	5.1	(2.6 - 9.2)	47	0.6	(0.3 - 0.9)
7.1 - 12	0			7	1.5	(0.1 - 4.6)
12.1 - 25	0			4	1.8	(0.1 - 7.7)
25.1 - 50	48	7	(2.8 - 16)	56	1.2	(0.7 - 1.9)
50.1 - 75	216	2.9	(2.1 - 3.9)	304	0.7	(0.6 - 0.9)
> 75	11	0.1	(-0.1 - 0.4)	11	0	

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent. 22 Samples for *L. longiflocosa* and 30 samples for *L. nuneztovari* where slope could not be identified were excluded.

25.1 - 50% slope of the general relief significantly higher than that in any of the other slope categories (Table 2.24, Annexe 16).

Univariate analysis also showed that *L. nuneztovari* abundance varied significantly with slope of the general relief ($F_{(35, 418)} = 1.63, p < 0.016$). *L. nuneztovari* abundance were significantly higher in the range 12.1 – 25% slope, than that in 50.1 – 75% ($z = -9.51, p < 0.001$), > 75% ($z = -7.48, p < 0.001$), and 3.1 – 7% ($z = -4.09, p < 0.001$) (Table 2.23). No significant differences were found when the range 12.1 – 25% was compared with the ranges 25.1 – 50% ($z = -1.49, p = 0.137$) and 7.1 – 12% ($z = -1.63, p = 0.103$). Multivariate analysis confirmed this association ($F_{(11, 390)} = 3.51, p < 0.001$), with *L. nuneztovari* abundance in sites with 12.1 – 25% slope of the general relief significantly higher than that all the other slope categories, except the category 3.1 – 7% slope (Table 2.25, Annexe 20).

2.3.6 Multivariate analyses of ecological determinants

All variables tested in the univariate analyses (excluding municipality, variables related with flora and the detailed habitat description) were tested for their ability to predict abundance within multivariate generalised linear models (GLIM), controlling for height of the trap and their position (edge / centre) in the *L. longiflocosa* model and only for position in the *L. nuneztovari* model.

2.3.6.1 *Lutzomyia longiflocosa*

Following model simplification the Minimal Adequate Model (MAM) for abundance of *L. longiflocosa* included fourteen significant variables: soil, temperature, temperature square, rainfall, rainfall square, altitude, altitude square, slope of the general relief, degree of protection from wind, number of tree strata, cover, litter cover, slope in the sampling site and habitat (Table 2.24). These explained 25% of the variance in abundance (Annexe 16). The goodness of fit of the model is illustrated by the plots of observed and fitted values (Annexe 17). Further support for the suitability of the model come from the random distribution of the Anscombe residuals plotted against the fitted values (Annexe 18) and the normal Quantil-Quantil plot of the Anscombe residuals (Annexe 19).

2.3.6.2 *Lutzomyia nuneztovari*

The MAM for abundance of *L. nuneztovari* included eleven significant variables, in decreasing order of importance: soil type, slope in the sampling site, slope of the general relief, number of tree strata, temperature, temperature square, habitat, distance to the nearest house, depth of partially decay litter, litter cover and rainfall (Table 2.25). This model explained 52.4% of the variance (Annexe 20). As for *L. longiflocosa*, the plots of observed against fitted values (Annexe 21), the plot of fitted values against their residuals (Annexe 22) and the normal Quantil-Quantil plot of the residuals (Annexe 23) confirmed the suitability and goodness of fit of the model.

Table 2.24 Ecological determinants for *Lutzomyia longiflocosa* abundance (raw number/CDC trap outdoors/night) identified by multivariate analysis.

Explanatory variable		coefficient	S. E.	z	p	95% C.I.		
						Min	Max	
Trap Location								
Height of the trap	1.5 m ^a							
	10 m	-0.183	0.227	-0.81	0.420	-0.629	0.262	
Interaction habitat and height of the trap	Traditional coffee vs 10 m	-1.048	0.345	-3.04	0.002	-1.725	-0.371	
	Semishaded coffee vs 10 m	0.913	0.948	0.96	0.336	-0.945	2.772	
Position of the trap (edge / centre)	edge ^a							
	centre	-0.308	0.381	-0.81	0.419	-1.055	0.439	
Interaction habitat and position of the trap	Traditional coffee vs centre	-0.447	0.501	-0.89	0.372	-1.429	0.535	
	Semishaded coffee vs centre	1.489	0.774	1.92	0.055	-0.029	3.007	
	Unshaded coffee vs centre	1.421	0.698	2.04	0.042	0.052	2.790	
Local determinants								
General habitat	Forest ^a							
	Traditional coffee	6.084	1.727	3.52	<0.001	2.698	9.469	
	Semishaded coffee	-10.199	2.188	-4.66	<0.001	-14.488	-5.910	
	Unshaded coffee	-10.585	5.700	-1.86	0.063	-21.756	0.586	
Slope in the sampling site (% range)	75.1 - 100 ^a							
	> 100	0.181	0.853	0.21	0.832	-1.491	1.853	
	0 - 3	-1.368	2.378	-0.58	0.565	-6.030	3.294	
	3.1 - 7	-10.301	1.989	-5.18	<0.001	-14.199	-6.403	
	7.1 - 12	-8.502	2.925	-2.91	0.004	-14.234	-2.769	
	12.1 - 25	-5.346	2.106	-2.54	0.011	-9.473	-1.219	
	25.1 - 50	-5.584	2.016	-2.77	0.006	-9.535	-1.632	
	50.1 - 75	-3.177	1.200	-2.65	0.008	-5.529	-0.825	
	Degree of protection from wind	Protected ^a						
		Partially protected	-0.080	0.350	-0.23	0.819	-0.766	0.606
Unprotected		4.042	0.939	4.3	<0.001	2.201	5.882	
Number of tree strata	4 ^a							
	0	-17.900	5.897	-3.04	0.002	-29.458	-6.342	
	1	-12.876	3.393	-3.79	<0.001	-19.526	-6.225	
	2	-6.585	2.772	-2.38	0.018	-12.018	-1.152	
	3	-6.255	2.209	-2.83	0.005	-10.584	-1.927	
Cover		-0.189	0.030	-6.32	<0.001	-0.247	-0.130	
Litter cover (% range)	81 - 100 ^a							
	20 - 40	-1.819	0.959	-1.9	0.058	-3.698	0.059	
	41 - 60	-0.259	1.372	-0.19	0.850	-2.948	2.430	
	61 - 80	-2.614	1.126	-2.32	0.020	-4.821	-0.406	
Regional determinants								
Altitude		0.253	0.034	7.44	<0.001	0.186	0.320	
Altitude square		-0.0001	0.00001	-6.87	<0.001	-0.0001	0	
Rainfall		0.097	0.043	2.25	0.024	0.012	0.181	
Rainfall square		-0.00003	0.00001	-2.46	0.014	-0.0001	0	
Temperature		-46.996	15.630	-3.01	0.003	-77.630	-16.361	
Temperature square		1.160	0.390	2.98	0.003	0.396	1.925	
Slope (% range)	25.1 - 50 ^a							
	50.1 - 75	-7.321	0.696	-10.52	<0.001	-8.684	-5.957	
	> 75	7.653	2.443	3.13	0.002	2.865	12.440	
	3.1 - 7	22.334	4.290	5.21	<0.001	13.927	30.742	
Soil type	MQA ^a							
	MQC	12.573	3.601	3.49	<0.001	5.516	19.630	
	MQD	20.459	3.827	5.35	<0.001	12.958	27.959	
	MQE	-0.999	1.245	-0.8	0.422	-3.439	1.441	
	MQG	1.588	2.297	0.69	0.489	-2.915	6.091	
	MQM	-31.610	5.532	-5.71	<0.001	-42.453	-20.768	
	MRA	4.708	1.524	3.09	0.002	1.722	7.695	
	PQF	-1.787	4.569	-0.39	0.696	-10.743	7.168	
	Intercept	232.977	152.731	1.53	0.127	-66.371	532.324	

^a Baseline category.

Table 2.25 Ecological determinants for *Lutzomyia nuneztovari* abundance (ln[sandflies+1]/CDC trap outdoors/night) identified by multivariate analysis.

Explanatory variable		coefficient	S. E.	z	p	95% C.I.	
						Min	Max
Trap Location							
Position edge or centre	edge ^a						
	centre	0.155	0.084	1.84	0.066	-0.010	0.319
Local determinants							
General habitat	Forest and traditional coffee ^a						
	Semishaded and unshaded coffee	-0.747	0.370	-2.02	0.044	-1.472	-0.021
Slope in the sampling site (% range)	25.1 - 50 ^a						
	50.1 - 75	-0.837	0.200	-4.19	<0.001	-1.229	-0.445
	75.1 - 100	-0.957	0.199	-4.8	<0.001	-1.348	-0.566
	> 100	-0.555	0.203	-2.73	0.006	-0.953	-0.156
	0 - 3	-1.225	0.286	-4.28	<0.001	-1.785	-0.665
	3.1 - 7	-0.746	0.321	-2.32	0.020	-1.376	-0.116
	7.1 - 12	-0.700	0.260	-2.7	0.007	-1.209	-0.191
	12.1 - 25	-1.370	0.216	-6.34	<0.001	-1.794	-0.946
	1 ^a						
	2	0.473	0.268	1.76	0.078	-0.053	0.999
Number of tree strata	3	-0.280	0.348	-0.8	0.421	-0.961	0.402
	4	-0.320	0.202	-1.59	0.113	-0.716	0.076
	0	0.662	0.246	2.69	0.007	0.180	1.143
Litter cover (% range)	41 - 60 ^a						
	61 - 80	-0.478	0.280	-1.71	0.088	-1.027	0.071
	81 - 100	-0.347	0.256	-1.36	0.175	-0.848	0.154
	20 - 40	-0.910	0.456	-2	0.046	-1.803	-0.017
Depth of partially decay litter		0.060	0.024	2.53	0.011	0.013	0.106
Distance to the nearest house		-0.001	0.000	-2.89	0.004	-0.001	0.000
Regional determinants							
Rainfall		0.001	0.000	2.56	0.01	0.000	0.002
Temperature		-2.533	0.763	-3.32	0.001	-4.029	-1.037
Temperature squared		0.067	0.019	3.44	0.001	0.029	0.105
Slope (% range)	12.1 - 25 ^a						
	25.1 - 50	1.527	0.320	4.77	<0.001	0.900	2.154
	50.1 - 75	0.951	0.249	3.82	<0.001	0.463	1.438
	3.1 - 7	-0.562	0.637	-0.88	0.378	-1.811	0.687
	7.1 - 12	1.183	0.329	3.6	<0.001	0.539	1.827
	MQE ^a						
Soil type	MQG	-0.832	0.341	-2.44	0.015	-1.501	-0.163
	MQM	1.384	0.661	2.09	0.036	0.089	2.679
	MRA	-0.937	0.234	-4.01	<0.001	-1.395	-0.479
	PQA	0.669	0.541	1.24	0.216	-0.391	1.730
	PQF	-1.830	0.486	-3.77	<0.001	-2.783	-0.878
	AQC	1.348	0.368	3.67	<0.001	0.627	2.068
	LXA	-2.920	0.319	-9.14	<0.001	-3.546	-2.294
	MLB	-1.359	0.303	-4.48	<0.001	-1.954	-0.765
	MQA	-0.356	0.159	-2.25	0.025	-0.667	-0.045
	MQC	-0.182	0.332	-0.55	0.583	-0.833	0.468
	MQD	-1.081	0.293	-3.69	<0.001	-1.654	-0.507
Intercept		23.367	7.408	3.15	0.002	8.847	37.886

^a Baseline category.

2.3.7 Geographical association between abundance of the two main sandfly species and cutaneous leishmaniasis incidence

Figure 2.18 maps the relation between the outdoor abundance of the two main sandfly species (caught by LTC) in the seven sampled municipalities in 1998 and CL mean annual incidence (CL records 1982 – 2004 from SSDH; excluding four year period when the records were not available) in Huila by municipality. Validation of the LTC as representative of the HLC, the direct measure of the risk of exposure to sandflies biting, was demonstrated by the positive association found between these two catching methods (section 2.3.3.3). Furthermore, LTC outdoors could be taken as representative of the general risk of exposure (both outdoors and indoors) to sandfly biting based on the apparent association between catches outdoors and indoors (Figures 2.4 and 2.5).

L. longiflocosa abundance had apparently a positive association with incidence of CL. This sandfly species presented its highest abundance in Baraya, 5.9 s/LT/n and Neiva, 7.2 s/LT/n, located within the epidemic area of Huila on the North-East of the Cordillera Oriental where the highest annual incidence of CL is recorded (Baraya, 215 x 100,000 and Neiva, 190 x 100,000) or in Algeciras, 7.0 s/LT/n, a contiguous municipality to the epidemic area with a mid incidence (9.1 x 100,000) of CL. It is important to note that the incidence in Algeciras was due to a small outbreak that occurred after the sandfly sampling, between the years 1999 to 2000, and that after the sampling the SSDH was aware of the potential high risk for CL in this municipality. *L. longiflocosa* was absent or had a low abundance in the sampled municipalities located outside the epidemic area where apparently there were no autochthonous cases of CL: Santa María, Iquira and Saladoblanco (Cordillera Central), and Garzón (South of the Cordillera Oriental).

The distribution of *L. nuneztovari*, in contrast, seems unrelated to CL incidence. The highest abundance of this species was found in Garzón (2.6 s/LT/n), which had no autochthonous cases of CL. *L. nuneztovari* presented its lowest abundance, 0.3, 0.5, and 0.6 s/LT/n, in the sampled municipalities with high or mid incidence of CL (Baraya, Neiva and Algeciras, respectively).

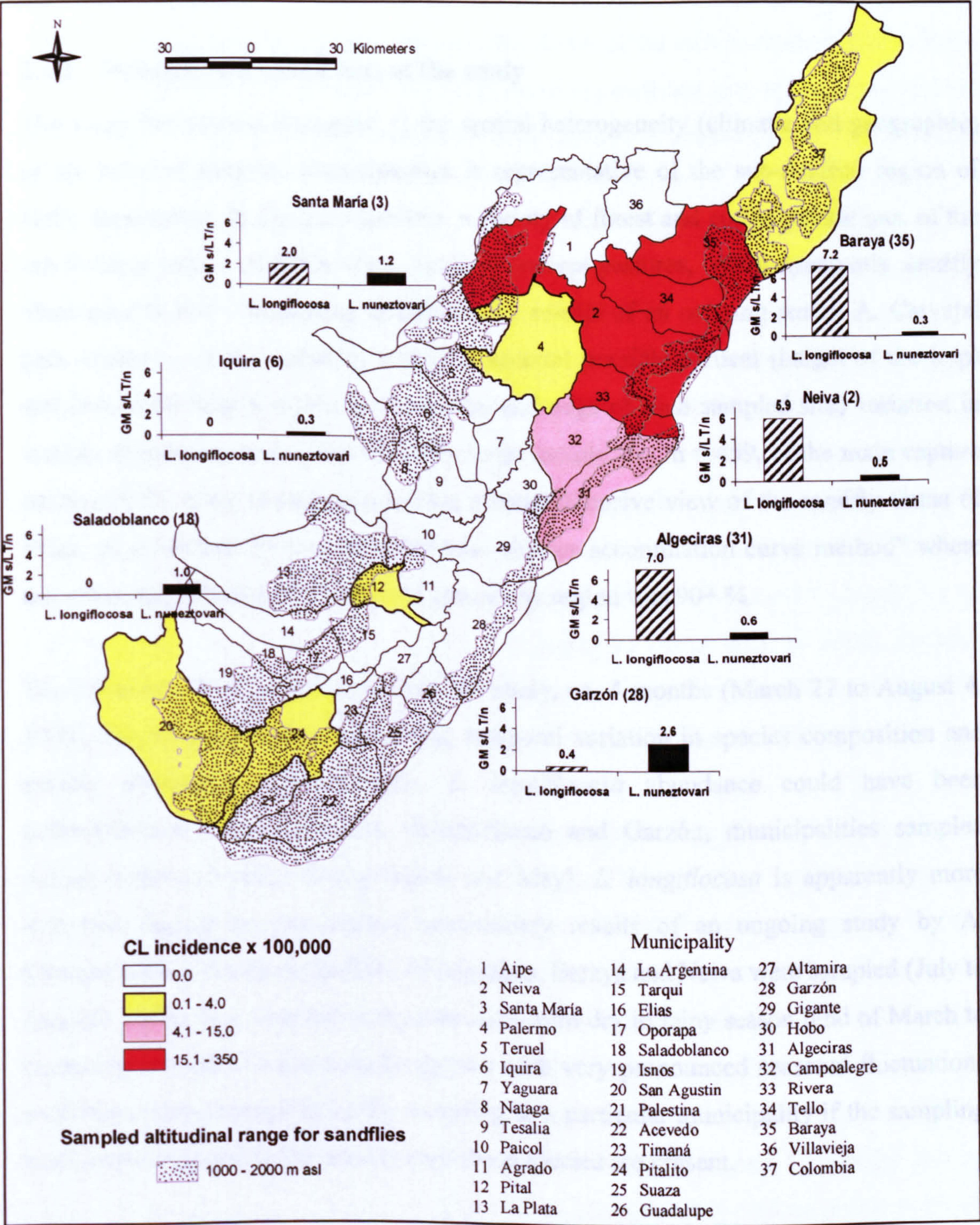


Figure 2.18 Association between the outdoor geometric mean (GM) abundance of *L. longiflocosa* and *L. nuneztovari* in the seven sampled municipalities in 1998, as measured by CDC light traps and cutaneous leishmaniasis (CL) annual incidence, 1982 - 2004 (excluding the period 1996 – 1999 where records were not available), by municipality.

2.4 DISCUSSION

2.4.1 Strength and limitations of the study

The study had several strengths: 1) the spatial heterogeneity (climatic and geographic) of the selected sampled municipalities is representative of the sub-Andean region of Huila department; 2) the main habitats, a variety of forest and coffee plantations, of the sub-Andean region of Huila were sampled, (except pastures, where apparently sandfly abundance is low – according to preliminary results of an ongoing study: A. Carvajal pers. comm.) ; 3) the sampling took into account possible vertical (height of the trap) and horizontal (trap position on the centre and edge of each sampled site) variation in sandfly abundance; and 4) the relatively large sample size, $n = 459$, of the main capture method (CDC traps outdoors) provided a comprehensive view of the sandfly fauna of Huila, as confirmed by the “sampled-base species accumulation curve method” where the estimated percentage of collected species by region was 90+ %.

The relatively short period of time of the study, ca. 4 months (March 27 to August 4, 1998), limited potentially confounding temporal variation in species composition and sandfly abundance between sites. *L. longiflocosa* abundance could have been underestimated in Santa María, Saladoblanco and Garzón, municipalities sampled during months of rainy season (April and May). *L. longiflocosa* is apparently more abundant during the dry season (preliminary results of an ongoing study by A. Carvajal), when the municipalities of Algeciras, Baraya and Neiva were sampled (July to August). Iquira was sampled in the transition from dry to rainy season (end of March to beginning of April). Some sandfly species with very pronounced seasonal fluctuations could have been omitted from the sampling in a particular municipality if the sampling was carried out outside the season where these species are present.

Another possible weakness was the reliance on LT catches, as LTs are a selective sampling method which only catch phototropic species. To deal with this weakness, additional catches using protected human bait and aspiration of resting sandflies from tree trunks were included. In fact, LT caught all the sandfly species detected by the other two methods (Table 2.5), and seven sandfly species were collected only by LT, the majority in low numbers. While the relative species composition of LT catches may differ from HL catches, *L. longiflocosa* dominated both, and it was reassuring to find a strong significant

positive correlation between *L. longiflocosa* outdoor abundance estimated by HLC and LTC. Of course, full knowledge of the sandfly fauna of the sub-Andean region in Huila should include (in future studies) additional sampling methods (e.g. sticky traps, Disney traps and Malaise traps) and sampling throughout the year to allow for seasonal variations.

2.4.2 Species composition, distribution and abundance

The relative low species richness (21 species, including 13 anthropophilic) of the sandfly fauna in Huila department agrees with the apparent poor sandfly fauna (in number of species) in the sub-Andean region of Colombia which is dominated by the *verrucarum* and *vexator* groups (Young 1979). In the present study the *verrucarum* group was the more diverse sandfly group with six species (*L. longiflocosa*, *L. nuneztovari*, *L. andina*, *L. columbiana*, *L. oresbia* and *L. pia*) accounting for 94.1% of all sandfly caught for all sampling methods. This group, probably originating in North-West of South America (Bejarano *et al.*, 2003a), includes many anthropophilic species which are suspected or are proven vectors of CL in the Andean region (Young and Duncan 1994).

It is difficult to compare the results of the present study with other studies because of differences in sampling methods, effort and duration of the sampling. In addition there are few regional studies of the sub-Andean sandfly fauna. With this caveat it appears that the diversity of the sandfly fauna of Huila is similar to that found in two states of Venezuela. In Mérida a study in 15 localities using several sampling methods [sticky traps in resting sites (StR), direct aspiration in resting sites (AR), Shannon traps (ShT) and HL] during approximately two years recorded 24 sandfly species (Añez *et al.*, 1988); and in Trujillo a study in 280 localities using AR and HL during five years found 17 sandfly species (Mogollon *et al.*, 1977). To my knowledge in the Colombian sub-Andean region there are no previous studies involving large areas at departmental level. Nevertheless, several studies carried out in small areas with coffee plantations sampled for more than one year seem to confirm the low diversity of the sandfly fauna of this region: Arboledas municipality (Norte de Santander department), where 17 sandfly species were collected by AR, HL, LT, ShT, Disney traps (DiT) and Malaise traps (MaT) (Alexander *et al.*, 1992); La Guaira (Valle del Cauca department), where five sandfly species were collected by StR, AR HL, LT, and ShT (Alexander *et al.*, 1995d); and Anolaima municipality

(Cundinamarca department), where seven species were collected by LT and ShT (Bello *et al.*, 2002). In highland (> 2000 m a.s.l.) areas of the Andes with a less structured vegetation (xerophytic vegetation) sandfly diversity apparently is further reduced. In the Peruvian inter-Andean valleys of Lampalla (Ayacucho department) and Purísima (Ancash department), where catches by LT, ShT, AR and HL were carried out, two and six sandfly species were found, respectively (Cáceres *et al.*, 2004; Villaseca *et al.*, 1993). In contrast, sandfly diversity seems higher in the lowlands rain forest areas, presumably due to the greater habitat complexity. For instance in Putumayo department (Amazon), the only previous study at regional level in Colombia, at least 42 sandfly species were detected amongst the 54 localities sampled using ShT, LT, HL and AT (Barreto *et al.*, 2000). Studies in smaller areas confirm this trend. In El Opon (Santander department), a CL focus located in the foothills of the Cordillera Oriental which surround the Mid-Magdalena valley, 27 sandfly species were recorded by LT and HL (Muñoz-Mantilla 1998). In Panama, a study in five lowland rain forest sites collected 33 sandfly species using AT (Christensen and Vasquez 1982).

There is no obvious explanation for the differences in sandfly diversity between the three main regions sampled in Huila. The lower number of sandfly species and overall abundance detected in the Colombian Massif could be associated with the lower temperatures present in the majority of this region. It may also be relevant that the hot Magdalena valley is a natural physical barrier for the sandfly fauna of the sub-Andean region of the Cordilleras Central and Oriental.

The overwhelming dominance of one species (*L. longiflocosa*) in the sampling area (particularly in and around the CL epidemic region of Algeciras, Neiva and Baraya) is an extreme example of the common pattern that most communities contain a few dominant species and many species that are relatively uncommon (Krebs 1999). The overwhelming dominance of *L. longiflocosa* is also a feature of the other three foci of CL in the sub-Andean region where *L. longiflocosa* is the suspected vector: Planadas, 99% of all catches (Cárdenas *et al.*, 1999) and Chaparral-San Antonio, 80% (Pardo *et al.*, 2006), both municipalities in Tolima department; and Abrego, 91%, in Norte de Santander (Cárdenas *et al.*, 2005). In the two Tolima foci (as in Huila), *L. longiflocosa* is also sympatric with *L. nuneztovari* and species of the *Helcocyrtomyia* subgenus. Other suspected vector species in the *verrucarum* group dominate in other parts of the

sub-Andean region, for example in Colombia: *L. columbiana* in Nariño department, (Montoya-Lerma *et al.*, 1999); *L. torvida* in Cundinamarca (Bello *et al.*, 2002); and *L. youngi* in Valle del Cauca (Alexander *et al.*, 1995d); in Bolivia: *L. nuneztovari* in Las Yungas (Torres *et al.*, 1998) and La Paz (Martinez *et al.*, 1999); and in Venezuela: *L. youngi* in Trujillo (Scorza *et al.*, 1984). Dominance of a single resistant species could be associated with the loss of a large proportion of original habitat and with severe habitat fragmentation (Myers *et al.*, 2000), both features characteristic of the sub-Andean region for a long time.

Finally, the present study contributed one new record of a sandfly for South America (*L. oresbia*), another for Colombia (*L. lerayi*) and nine new records for Huila department: the former two plus *L. andina*, *L. ayrozai*, *L. carpenteri*, *L. erwindonaldoi*, *L. shannoni*, *L. scorzai*, *L. sp of pichinde*.

2.4.3 Impact of forest replacement by coffee and the method of coffee cultivation on the species composition and abundance of anthropophilic sandfly fauna

Reduction in biodiversity (species richness) and abundance from forest and /or traditional coffee to intensive coffee plantations has been demonstrated for many animal communities: insects (Perfecto *et al.*, 1996; Borkhataria 2000), amphibians, reptilians, mammals (Borrero 1986), and birds (Robers *et al.*, 2000; Borkhataria 2000; Borrero 1986). In this study, strong differences in species richness and abundance of anthropophilic sandfly species were also found between forest (12 species and high sandfly abundance) and coffee plantations (≤ 7 species and lower sandfly abundance), regardless of method of coffee cultivation. Coffee plantations had broadly similar anthropophilic sandfly species (low β diversity values), but some differences suggest a descendent gradient in diversity and sandfly abundance (especially for *L. longiflocosa* – see 2.4.5) from traditional coffee growing, to semishaded coffee and unshaded coffee. Traditional coffee and semishaded coffee growing shared practically the same sandfly fauna ($\beta = 1$); but the sandfly fauna in traditional coffee was relatively different from that in unshaded coffee ($\beta = 3$). However, these patterns were not replicated by the the UPGMA cluster analysis of the anthropophilic sandfly fauna, which (as in a previous study in Colombia: (Alexander *et al.*, 2001)) were not clustered by general habitat types. Some differences may have been masked by migration of adult sandflies from nearby

forests (e.g. for host seeking). This is supported by two facts: (a) the majority of sampled sites were close to forest fragments, usually within 200 m, i.e. within the reported dispersal range of sandflies in the sub-Andean region (Alexander and Young 1992); and (b) all sandfly species found in coffee plantations, except *L. gomezi* (found in only one site with eight specimens), were a subset of those found in forest.

Forest, with few exceptions, is the characteristic pristine habitat for most sandflies of the sub-Andean region, where highest sandfly species diversity is expected. Primary forest, specifically rain forest, is also expected to have a high number of co-dominant species (Ready *et al.*, 1983), with selected species increasing in dominance and relative abundance following habitat modification (Mouchet *et al.*, 1994). In Huila, no fully intact forest was detected to test the sandfly fauna in the absence of degradation; but it is noteworthy that dominance of a single species, *L. evansi*, has been reported in a pristine tropical dry forest in Colombia (Travi *et al.*, 2002), indicating that the typical pattern may not always apply to forests in the sub-Andean region, which experience relatively strong climatic variations (e.g. rainfall and temperature).

The capacity of a sandfly to adapt depends on the similarity of the “new habitat” to its natural ecological niche (Peters and Killick-Kendrick 1987), so the relatively large difference in diversity between traditional coffee plantations and forest ($\beta = 7$) was unexpected. This may reflect the deteriorated structure of the sampled traditional coffee plantations (section 2.4.4) or possibly be an artefact of the relatively low sample size for traditional coffee sites (11 vs. 24) – which could not be sampled at all in two municipalities (Garzón and Iquira). A previous study in two regions of Colombia also reported species richness and total sandfly abundance to be higher in traditional coffee (12 species in each region) than in neighbouring intensive unshaded coffee plantations (8 and 10 species, respectively) (Alexander *et al.*, 2001). A study in Brazil found four sandfly species in traditional coffee compared to two in nearby intensive coffee plantations (Alexander *et al.*, 2002). Regarding forest degradation, in Colombia eleven sandfly species were found in intact primary forest (tropical dry forest) compared with only seven in a nearby degraded forest (Travi *et al.*, 2002); and in Brazil, a primary forest harboured 21 sandfly species, compared to only 14 in a nearby secondary forest (Luca de *et al.*, 2003).

2.4.4 Suspected sandfly vectors and incriminatory evidence

From the 13 anthropophilic sandflies species identified in the sub-Andean region of Huila department, six (*L. longiflocosa*, *L. nuneztovari*, *L. columbiana*, *L. gomezi*, *L. lichi*, and *L. longipalpis*) are suspected or proven vectors of leishmaniasis in Colombia or other countries of the Andean region. The following discussion focuses mainly on *L. longiflocosa* and *L. nuneztovari*, the two most common species. The remaining anthropophilic species apparently do not have high epidemiological importance because of their very low numbers.

L. longiflocosa appears to be the main vector of CL in Huila department, at least within the epidemic area, and *L. nuneztovari* may be a relatively unimportant secondary vector. The reasons for these conclusions are:

- (1) *L. longiflocosa* is the anthropophilic species with the highest dominance in Huila department (89% of all sandflies collected against 4.6% for *L. nuneztovari*).
- (2) *L. longiflocosa* is more anthropophilic than *L. nuneztovari*. This is suggested by the higher ratio HL : LT = 2.4 : 1 and 0.83 : 1, respectively; and the higher GM human landing rates outdoors, up to 23 f/p/40 min (i.e. 35 f/p/h) vs 1.1 f/p/40 min (i.e. 1.7 f/p/h), respectively (Annexe 9).
- (3) *L. longiflocosa* is more endophagic than *L. nuneztovari* (ratio LT indoors / LT outdoors = 0.64 and 0.5, respectively).
- (4) There is a strong geographic association between the municipalities where *L. longiflocosa* was present and the municipalities where CL has been reported. In contrast, *L. nuneztovari* is not associated with the presence of CL (Figure 2.18, Annexes 7 - 10).
- (5) The municipalities with the highest abundance of *L. longiflocosa* outdoors (Figure 2.4) are also the municipalities with the highest incidence for CL during the period 1982 - 2004 (Figure 2.18). In contrast, the municipalities with the highest abundance of *L. nuneztovari* (Figure 2.5) are municipalities where there are no autochthonous cases of CL (Figure 2.18).

L. longiflocosa has been identified as a suspected vector in four foci of CL in municipalities of the sub-Andean region surrounding the Magdalena valley: (a) Chaparral and San Antonio (Tolima department), located on the Cordillera Central, where between 2002 – 2003 the biggest epidemic of CL in Colombia was recorded

(Pardo *et al.*, 2006); (b) Planadas, in the same Cordillera in Tolima, where a small outbreak took place in 1998 (Cárdenas *et al.*, 1999); (c) Abrego (Norte de Santander department) located on the Cordillera Oriental where another small outbreak occurred between 2001 - 2004 (Cárdenas *et al.*, 2005); and (d) Algeciras, located next to the southern border of the epidemic area of the present study, where a small outbreak took place in 1999 (Carvajal A., personal communication). Additional incriminatory evidence from *L. longiflocosa* comes from a competence study, mentioned in Chapter 1, which demonstrated experimental infection of *L. longiflocosa* with *Le. braziliensis* and transmission of this parasite to a host by infected females (Santamaría *et al.*, 1998).

While the reported experimental infection of *L. nuneztovari* with *Le. braziliensis* in Colombia provides evidence of the vectorial competence of this species (Santamaría *et al.*, 1999), the abundance of *L. nuneztovari* throughout Huila is remarkably low (reaching a maximum GM landing rate of 2 f/p/h) compared to that in Cajuata and the Yungas in Bolivia (reaching an arithmetic mean up to 44 f/p/h (Le Pont *et al.*, 1989a)) where this species was previously incriminated as a vector of *Le. amazonensis* and *Le. braziliensis* by the detection of natural infections (Martinez *et al.*, 1999; Torres *et al.*, 1998).

Although there is overwhelming circumstantial evidence pointing to *L. longiflocosa* as the main vector of CL in the sub-Andean mountainous region of Huila department, final confirmation still requires the detection of wild females naturally infected with *Le. braziliensis*.

2.4.5 Ecological determinants for the two main sandfly species

L. longiflocosa showed a discontinuous distribution along the mountainous area of the sampled municipalities. It appears that the distribution of *L. longiflocosa* in Huila department covers the major part of the West side of the Cordillera Oriental and a narrower area towards the North, on the East side of the Cordillera Central. This information enlarges to fifteen municipalities the known distribution of this species endemic of Colombia, restricted until now to eleven municipalities in four departments located on the sub-Andean area surrounding the Magdalena valley: Huila [two municipalities (Ferro *et al.*, 1998a)]; Tolima [seven municipalities (Pardo *et al.*, 2006;

Young and Duncan 1994; Bejarano *et al.*, 2003b; Sierra *et al.*, 2000; Cárdenas *et al.*, 1999)(unpublished data, Laboratorio de Entomología, INS)]; Cundinamarca [one municipality (Ferro *et al.*, 2005)]; and Norte de Santander [one municipality (Cárdenas *et al.*, 2005)]. In contrast, *L. nuneztovari* presented a continuous distribution along the seven sample municipalities. So *L. nuneztovari* has a more widespread distribution than *L. longiflocosa* suggesting a more generalized ecological niche. This is consistent with the widespread geographical distribution of *L. nuneztovari* in Colombia (Bejarano *et al.*, 2003b) and in Central and South America, ranging from Guatemala to Bolivia (Young and Duncan 1994).

For both *L. longiflocosa* and *L. nuneztovari*, regional plus local determinants (in total 10 and 11, respectively) were found associated with sandfly abundance by multivariate analyses. The determinants of *L. longiflocosa* abundance are also potential risk factors for CL in Huila. Hence, the aggregated character of these determinants appears to explain both the aggregated distribution in *L. longiflocosa* abundance as well as the aggregation pattern also observed for CL at municipality and village scales (Chapter 1, section 1.2).

However, it should be stressed that the explanatory power of the models was relatively low, particularly the MAM for *L. longiflocosa* which explained only 25% of the variance in sandfly abundance. This may be because key variables were not recorded, or because of inaccuracy in the measurements of the environmental variables tested and/or sandfly abundance at the sampled locations; e.g. climate and land cover data could be replaced by high resolution remotely sensed data, and other proxy determinants could be replaced by the true potential determinants (e.g. replace “degree of protection” by wind speed). With these caveats, there follows a description of the characteristics of sites with the highest risk (“hot spots” for sandflies) for both *L. longiflocosa* and *L. nuneztovari* with a brief discussion of each ecological determinant contributing to high risk.

At local scale, the highest abundance of *L. longiflocosa* was found in sites with relatively high slope (75 - 100%), protected from the wind, where the habitat was forest with the following features: four tree strata, high cover ($\geq 60\%$), and high litter cover ($> 80\%$). At regional scale, these sites were located in areas with moderate slope (25 -

50%), where temperature is approximately between 18°C - 20°C, rainfall between 1000 – 1800 mm, altitude between 1500 – 1700 m a.s.l., and soil of type MQA.

With respect to *L. nuneztovari*, at local scale, high abundance of this species was found in sites with moderate slope (25 – 50%), where the habitat was forest or traditional coffee plantations with the following features: one tree stratum, with moderate litter cover (41 - 60%) which has a relatively deep layer (> 3 cm) of partially decayed litter, and where houses were close (< 200 m) to the sampling site. At regional scale these sites were located in areas with a relatively low (12 - 25%) slope, where temperature is between 19°C – 23°C, rainfall between 1600 - 1800 mm, and soil of type MQE.

Among local determinants, *L. longiflocosa* abundance was significantly higher in forest, followed by traditional coffee plantations and the lowest abundance was in intensive unshaded and semishaded coffee plantations. *L. nuneztovari* abundance presented a similar pattern with the highest abundance in forest and the lowest in intensive unshaded coffee plantations. However, while both species have their highest abundance in forest or forest-like (e.g. traditional coffee) habitats, the features of these habitats are different for each sandfly species. *L. longiflocosa* appears adapted to a lesser modified forest (several tree strata with high cover and litter cover), while *L. nuneztovari* seems to be adapted to a higher modified forest (only one tree stratum with moderate litter cover).

As discussed above, differences in sandfly abundance between habitats could be explained by the strong changes in physiognomy and structure with a decreasing trend in flora and structure complexity from forest to intensive unshaded coffee. Traditional coffee plantations had only six plant species in common with forest (*Inga culangana*, *Inga macrophylla*, *Ficus insipida*, *Bambusa guadua* and *Cupania americana*) and the differences in structure were notable (traditional coffee sites have less tree strata, cover, and litter). These differences may affect the survivorship of both adult and immature sandflies. The lower abundance of *L. longiflocosa* in semi-shaded coffee (0.5 s/LT/n) than in unshaded coffee plantations (1.9 (s/LT/n) was unexpected, but is probably due to bias: 90% of the sites classified as semi-shaded coffee were in municipalities where *L. longiflocosa* abundance was generally low or where this species was absent (i.e. Santa María, Iquira, Saladoblanco and Garzón).

In general, the results in Huila are consistent with more recent observational studies of *L. longiflocosa* ecology. In a CL focus in Tolima department, indoor abundance (CDC light traps) of *L. longiflocosa* was apparently higher in houses surrounded by either traditional coffee plantations or a mixture of forest/traditional coffee than by unshaded coffee or mixed forest/pasture. However, *L. longiflocosa* abundance in houses surrounded by forest was lower than in houses surrounded by traditional coffee or by mixed forest/traditional coffee (Pardo *et al.*, 2006).

From all the physiognomic-structural features of forest it seems that trees have the main effects on sandflies, because trees have been related with sandfly abundance both directly [cover (Rutledge and Ellenwood 1975a), buttresses (Christensen and Vasquez 1982) and holes (Comer *et al.*, 1993)] and indirectly [litter (Rutledge and Ellenwood 1975a; b)]. Shade (cover) given by trees modulates microclimatic conditions: rainfall, temperature and humidity in the forest floor and provide protection from wind. Trees also produce and stabilize most of the litter (Rutledge and Ellenwood 1975a). Hence, it was not unexpected that in the present study tree strata, cover by trees, litter cover and depth of litter were all related with *L. longiflocosa* and *L. nuneztovari* abundance. The association of both species with slope of the sample site could be explained by the effect that slope has on the pattern of distribution of the forest litter, its composition, texture and depth (Rutledge and Ellenwood 1975c).

Although flora and detailed habitat classification according to physiognomic-structural features were not included within the models for the multivariate analysis, these variables require a short comment. The failure to detect any significant associations between specific plant species and the distribution of either *L. longiflocosa* or *L. nuneztovari* is presumably due the relatively high diversity of plant species of the sub-Andean region in Huila (Rangel and Garzón 1995) and the strong geographical association with most plant species. In contrast, the classification of habitats according to physiognomic-structural features successfully distinguished the specific types of forest which harboured the highest abundance of both sandfly species and those forest types which appeared unsuitable for sandflies. Sandfly abundance was even shown to vary in relation to the structural features of intensive unshaded coffee. For example, *L. longiflocosa* was 3x more abundant in habitat 11 (evergreen high shrub) characterised

by coffee plants > 2 m height than in habitat 2 (evergreen low shrub), where coffee plants were < 2 m height.

Vegetation characteristics in different localities of Huila (as elsewhere) are strongly influenced by climate and soil, and as throughout the Andes environmental characteristics are all closely associated with altitude. Hence, although climate, soil and altitude were all shown to impact on the distribution of *L. longiflocosa* (and to some extent *L. nuneztovari*), it is unclear what are the causal links for each of these associations. For example, soil type may have an impact on sandfly larval breeding sites or it may simply reflect variation in the fauna and flora of a locality which would impact on sandfly adult survival. There are indeed only a few published studies relating the abundance or presence of sandflies vectors with soil type, *P. orientalis* in the Sudan (Elnaiem *et al.*, 1998) (Thompson *et al.*, 1999), and none in Latin America prior to this study.

The confirmed negative association between distance to the nearest house and *L. nuneztovari* abundance, an association also suggested for *L. longiflocosa* by the univariate analysis, could be explained by a low abundance of hosts in the sampled habitats. Sandflies are thus more likely to forage in the domestic environment where potential hosts (i.e. humans and domestic animals) are clustered, and are relatively easy to bite (resting/asleep during peak sandfly activity).

Degree of protection from wind (as a proxy of wind speed) also affected the two species in different ways. *L. longiflocosa* seems more affected by wind as its abundance was favoured by protected sites. In contrast, *L. nuneztovari* seems unaffected by wind because its abundance was unaffected by the degree of protection from the wind (indicating it is a more wind-tolerant species). It is known that wind is a climatic factor which affects the activity of adult sandflies (Killick-Kendrick *et al.*, 1985). Highest sandfly abundance occurs with wind speed < 0.3 m/s (Roberts, D.M. 1994) and strong winds are unfavourable for sandflies (Rioux *et al.*, 1997). Mean wind speed in the Colombian coffee area, measured at 10 m height, is 10 km / h (i.e. 2.8 m / s), with maximum speed of 30 km / h (i.e. 8.3 m / s). Within the study area this factor gains high importance because strong winds occur during the second dry season (July and August) of the year (Guzmán and Gómez 1995), when *L. longiflocosa* seems to be most

abundant (Chapter 5, section 5.3.2.3). Hence, sites protected from wind are significantly preferred by *L. longiflocosa*.

At regional level, temperature was the only climatic determinant of *L. longiflocosa* with a well defined environmental gradient, with the greatest sandfly abundance (optimum range) between 18 – 20°C, a minimum limit of tolerance \approx 16°C, and a maximum limit of tolerance \approx 24°C. However, the possibility of confounders can not be discounted as the extreme temperatures were only detected in municipalities where *L. longiflocosa* was absent or had the lowest abundance (Iquira, Saladoblanco and Garzón), irrespective of temperature. In contrast, the distribution of *L. nuneztovari* was less dependent on temperature. Its distribution was bimodal with peaks of abundance in the ranges 19 – 20°C and 22 – 24°C, and no clear limits of tolerance, within the range sampled.

The distribution of both sandfly species appear to be dependent on rainfall. *L. longiflocosa* abundance was bimodal with two peaks: 1200 - 1400 mm and 1600 – 1800 mm with maximum limit of tolerance apparently near to 2200 mm and lowest limit not detected. The absence of both sandfly species in the range 1800 – 1999 mm (Figures 2.14 and 2.15) could be explained by confounders because this range was only found in Iquira municipality, where (irrespectively of rainfall) *L. longiflocosa* was absent and *L. nuneztovari* presented the lowest abundance. Similarly, for *L. longiflocosa*, the apparent reduction in the range 2000 – 2199 mm could be biased because this range was only sampled in municipalities with low abundance or absence of this species (Santa María and Iquira). In contrast, *L. nuneztovari* presented an apparent unimodal distribution which peaked in the range 1600 – 1800 mm, with a low limit of tolerance around 1000 mm. The upper limit was not detected.

As for many Andean sandfly species, the niche of *L. longiflocosa* within Huila was defined by a relatively narrow altitudinal range (in Huila) with the optimum range between 1500 – 1700 m a.s.l. (reflecting the region of highest risk of disease transmission), a minimum limit of tolerance around 900 m a.s.l. and a maximum limit of tolerance not detected. In contrast, *L. nuneztovari*, seems a more generalist species with no strong variation within the altitudinal range sampled.

There are no other studies directly comparing climatic variables and altitude with the abundance of *L. longiflocosa*. However, it is pertinent to compare the results of this study with the climatic and altitude features of other locations where this species has been studied, e.g. in the foci of Abrego, Norte de Santander department (Cárdenas *et al.*, 2005), Planadas (Cárdenas *et al.*, 1999) and Chaparral-San Antonio, both in Tolima department (Pardo *et al.*, 2006). In all three locations the altitude and temperature characteristics are consistent with the preferred niche in Huila. However, it is notable that the Tolima focus has relatively high rainfall (> 2000 mm), in contrast to the Huila populations (where the upper limit of tolerance is apparently ca. 2200 mm.). One possible explanation is that the Tolima populations have adapted to higher rainfall since being geographically isolated from the Huila populations by the Magdalena valley. Future genetic studies could shed light on such speculations.

2.4.6 Conclusions

CL foci in Huila are limited to the north east municipalities on the Cordillera Oriental. The most probable vector of CL in Huila seems to be *L. longiflocosa* based on its high dominance, anthropophily, endophagy and geographical overlap with the area of highest CL incidence in Huila department. *L. nuneztovari* could play, at best, a secondary vectorial role because of its low abundance and lack of association with the geographical distribution of CL. The endophagic behaviour of *L. longiflocosa* suggests that indoors transmission may be important. Nevertheless, this should be confirmed by more detailed studies.

For *L. longiflocosa*, the narrower, and better defined, gradient of altitude and temperature, its apparent preference for well structured and complex forests (several tree strata, high cover and high litter cover) and dependence on sites protected from wind, indicates that this species has a narrow ecological niche. In contrast, for *L. nuneztovari*, its less well defined ranges of temperature and rainfall and apparent tolerance to highly disturbed forest (few tree strata with low litter) and apparent lack of requirement for protection from wind, suggest that this species has a wider ecological niche, making it more of a generalist.

To my knowledge this is the first study where sandfly diversity and abundance is compared taken into account simultaneously forest and methods of coffee cultivations. The relatively high *L. longiflocosa* and *L. nuneztovari* abundance in traditional coffee plantations support the hypothesis that these sandflies are “completely adapted” to this type of habitat (i.e. they can find all the requirements for their life cycle: sources of food and sites for resting, mating and breeding). Similar adaptation of a sandfly species to a new habitat was described in Brazil where *L. flaviscutellata*, a sandfly proper of secondary forest, became adapted to tree plantations (Ready *et al.*, 1983). Traditional coffee plantations are characterized mainly by the presence of trees which appears one of the main factors related with sandfly abundance (section 2.4.5). As in forest, trees in traditional coffee plantations regulate temperature, water contents, help to keep soil fertility and reduce wind speed (Moguel and Toledo 1999; Jaramillo 1976) - conditions which, in addition to the resting sites provided by their trunks, favour sandfly survival.

In contrast, the lower abundance of *L. longiflocosa* and *L. nuneztovari* in the two intensive coffee plantations could be explained by a “partial adaptation” to these habitats where only adults of the two sandflies species are able to use these habitats for foraging activities (e.g. host finding and sources of sugar meals). Complete adaptation to intensive coffee habitats, particularly to unshaded plantations seems unlikely because the strong structural changes caused by the lost of the tree strata make this habitat apparently unsuitable for sandflies (Chapter 1, section 1.3). One of the main difficulties for sandflies to adapt to unshaded coffee plantations could be the conditions for breeding. Soil in unshaded coffee sites is exposed to large fluctuations in temperature, humidity and rain, not least due to the limited leaf litter coverage (formed exclusively of coffee leaves) (Table 2.13). Organic material in intensive unshaded coffee is reportedly about half of that found in either forest or traditional coffee habitats (Chamorro *et al.*, 1994).

Nevertheless, future adaptation of either *L. longiflocosa* or *L. nuneztovari* to intensive unshaded coffee cannot be completely discounted. For example, in Trujillo, Venezuela, *L. youngi*, another species of the *verrucarum* group, was still present 12 years after a traditional coffee plantation was replaced by ground crops, where almost all sources of shade were removed (Rojas *et al.*, 2004). Indeed, it is notable that the dramatic replacement of traditional coffee in Colombia during the last thirteen years by

intensified plantations [percentage of intensive plantations in 1970 = 0.2%; 1980 = 34%; and 1997 = 70%; total coffee area = 1,067,000; 1,004,000 and of 869,000 ha, respectively (Guhl 2004)] seems to have had no negative impact on CL incidence. CL has actually increased notably during this period (Chapter 1, section 1.2). This could be explained by the persistence of small fragments of forest in close proximity to the coffee plantations - big enough to harbour high sandfly populations. In addition, the higher demand for human labour in the intensive plantations may have led to an overall increase in the human population at risk, at least during harvest periods.

3 HOUSE RISK FACTORS

3.1 INTRODUCTION

3.1.1 Overview on risk factors

Identification of risk factors (where does the transmission occur? when does it occur? and who are more likely to get infected?) are key points for developing any adequate programme of control and prevention. Identification of the places of transmission (indoors, peridomestic environment, away from the house) defines the type and feasibility of control measures. Although all the components of the epidemiological cycle could be involved as determinants of transmission (or risk factors), the entomological component seems more important, particularly those factors related to the exposure to sandfly-vector biting such as sandfly habitats, endophagy / endophily, diurnal rhythms and seasonal variations. Davies *et al.* (2000b) summarized the main studies on these aspects. Attention also has been given to those risk factors related to man (demography, behavioural patterns, house design and domestic animals).

The transmission of CL changed in the second part of the last century, mainly due to human intervention which has consequently modified the risk factors for the disease. Traditionally CL in Latin America has been related to behavioural factors which increase the human-vector contact in the forest (extra-domiciliary transmission). So entering the forest after sunset, hunting, lumbering and collecting other products (e.g. chewing gum) were the major risk factors for transmission and males were identified as at higher risk (Ward 1977; Dedet *et al.*, 1989). A similar pattern was considered to occur in all endemic areas of CL in Colombia (Ministerio de Salud de Colombia 1994). One of the main changes which humans have caused during recent decades has been deforestation and it was suggested that this would lead to a reduction of CL incidence. However, it frequently led to domestication of transmission throughout Latin America (Walsh *et al.*, 1993). This increase in disease incidence suggested that following deforestation and colonization, parasites and sandflies are capable of adapting to the new environmental conditions (Lainson 1988; Grimaldi and Tesh 1993; Walsh *et al.*,

1993). This domestication in transmission has switched the risk factors from places away from the houses to peridomestic and indoor environments. Nowadays there are frequent epidemiological reports demonstrating a wider age distribution and no gender difference (Desjeux 2001).

3.1.2 Sandfly risk factors

The first step prior to studying the entomological risk factors is to identify the sandfly vectors. Until recently, this task has typically been carried out using biological criteria of incrimination (Killick-Kendrick 1990): 1) anthropophily of the sandfly species, and its contact with disease reservoirs (for zoonotic diseases), 2) isolation of the same parasite species from sandflies and humans, 3) experimental infection of sandflies with the parasite species and transmission of the parasite by sandfly biting a susceptible mammal host, and 4) geographical overlap of vector and parasite. Because it is frequently difficult to meet all these criteria, especially the second [as sandfly infections are very low (usually less than 1%)], an additional indirect statistical approach can be taken. It is the regression analysis of the spatial relationship between the abundance of the suspected sandfly species and prevalence and / or incidence of CL (Davies *et al.*, 1997). This method has been used successfully in the incrimination of sandfly species as vectors of CL in Colombia, Peru and Venezuela (Muñoz-Mantilla 1998; Davies *et al.*, 1997; Feliciangeli and Rabinovich 1998).

Until now, to my knowledge, three quantitative Latin American studies have been carried out to identify risk factors for sandfly abundance – all, specifically, cross-sectional studies. The first, was carried out on Marajo island, Brazil, where fifteen surrounding environmental and house features were tested against the indoor abundance of *L. longipalpis*, the vector of VL, in 158 houses (Quinnell and Dye 1994a); as a result three variables (two house features and one domestic animal) were significantly correlated with sandfly abundance. The second study, carried out in the CL focus of Opon, in Colombia, tested five types of surrounding vegetation, at different distances, as potential determinants of indoor abundance of seven anthropophilic sandfly species in 114 houses (Muñoz-Mantilla 1998); this author found that three sandfly species were positively correlated with the percentage of cover of a specific type of crop around the houses. The last study was carried out in three rural areas in Brazil, where 16 variables

were tested as possible predictors for *L. whitmani* abundance inside and outside 196 houses; the results showed that four variables (three house features and one domestic animal) were correlated with the proportion of sandflies entering the house and five (two surrounding habitat features and three domestic animals) were related with the peri-domestic sandfly abundance (D. Campbell-Lendrum, personal communication).

3.1.3 CL risk factors

Several risk factor studies have been carried out at household level in Latin America, mainly using comparative analysis of incidence and prevalence in case-control, cohort, and cross-sectional studies. Few studies have included direct measures of entomological variables within the potential risk factors.

In the Andean region (Andes and its foothills > 500 m a.s.l.), four relevant studies should be mentioned. The first was a case-control study carried out in Lima, Ancash, and Piura, Perú, located in inter-Andean valleys above 2000 m a.s.l., where the remaining vegetation was low shrubs and where *L. peruensis*, *L. verrucarum* (in the first two places) and *L. ayacuchensis* (in the third) are the vectors of *Le. peruviana* (Llanos-Cuentas 1994). This author found three factors related to house features, seven related to the surrounding environment and only one related to occupation (in the extra-domicile). The second study was part of the above mentioned study of Muñoz-Mantilla (Muñoz-Mantilla 1998), carried out in the foothills of the West side of Cordillera Oriental of Colombia, altitude between 400 - 600 m a.s.l., in a highly deforested area covered mainly by cacao plantations, disturbed rain forest and pastures where *L. trapidoi* is the vector of *Le. panamensis*. This author found that an environmental feature surrounding the house (one type of vegetation) was correlated with prevalence of CL at household level, as well as at community level (three types of vegetation). The third study was a cross-sectional study, carried out in the North West of Pichincha, Ecuador located between 500 - 1800 m a.s.l., with a vegetation of Pacific rain forest where presumably *L. trapidoi*, *L. gomezi* and *L. hartmanni* are the vectors and *Le. guyanensis* and *Le. panamensis* were identified as the main parasites which caused the disease (Armijos *et al.*, 1997); The authors found that age (less than 5 years), gender (male), and house features were significant risk factors for CL. The fourth study, another cross-sectional study, was carried out in the Amazon plain and its contiguous

Andes foothills in Alto Beni and Beni, Bolivia (Alcais *et al.*, 1997), with altitude up to 700 m a.s.l. and vegetation of tropical rain forest, where *L. yucumensis*, *L. carrerai* and *L. llanosmartinsi* are the vectors of *Le. braziliensis*. The study compared the prevalence of CL in two populations (native and migrant). They found that gender (male), type of population; occupation (in the extra-domicile) and home-forest distance were significant risk factors.

The contrasting results of these studies illustrate the high complexity of transmission of CL in the Andean region which makes it difficult to identify common patterns. Nevertheless, it seems that in this region an important part of the transmission is indoors, except in Alto Beni and Beni (Bolivia) where the main components seemed located far away from the house. This trend to intra-domiciliary transmission could be related to the deforestation process which in this region has been carried out since pre-Spanish time, and could lead to the adaptation of some sandfly species to the anthropic environment. Taking into account the apparently large number of CL foci in the Andean region and its high ecological variability, it is clear that there are many foci of CL which should be investigated before obtaining a good understanding of the transmission and risk factors involved in the disease. It is also clear that the choice of control measures will vary in the Andes according to the local risk factor patterns: one size will definitely not fit all.

On the other hand, outside the Andean region, in areas where relatively large areas of primary forest are still present, i.e. the Amazon and Pacific rain forests, transmission apparently continues to follow the traditional pattern, with risk factors related to contact with the forest, away from houses (Weigle *et al.*, 1993).

3.1.4 Outline and rationale

The present work describes a house-based cross-sectional study focusing on the identification of intra-domiciliary and peri-domiciliary risk factors for CL and the generation of incriminatory evidence for the two suspected sandfly vectors. The study is described in three main sections. The first section concerns sandfly risk factors. This includes (1) a description of the species composition, relative abundance and spatial distribution of the sandfly population, and (2) a description of the risk factors for

L. longiflocosa and *L. nuneztovari*. The second section investigates the risk factors for CL prevalence, and includes a comparative description of CL epidemiology within the study villages. The third section presents evidence for vector incrimination: by spatial correlation between the indoor abundance of the two suspected sandfly vectors and CL prevalence.

3.1.5 Objectives

1) To identify determinants of local variation in indoor CL sandfly vector abundance (*L. longiflocosa* and *L. nuneztovari*) in Huila department.

Specific objectives:

1.1) To identify house features which are associated with *L. longiflocosa* or *L. nuneztovari* indoor abundance.

1.2) To test for any correlation between host abundance (humans and/or domestic animals) in and around houses with *L. longiflocosa* or *L. nuneztovari* indoor abundance.

1.3) To identify surrounding habitat features which are associated with *L. longiflocosa* or *L. nuneztovari* indoor abundance.

2) To identify household risk factors for CL in Huila department.

Specific objectives:

2.1) To describe the age prevalence distributions of CL (by gender) within the study area.

2.2) To identify demographic risk factors for CL.

2.3) To identify house features which correlate with CL prevalence.

2.4) To test for any correlation between host abundance (humans and/or domestic animals) in and around houses with CL prevalence within households.

2.5) To identify surrounding habitat features which are associated with CL prevalence within households.

3) To test for any household correlation between indoor abundance of *L. longiflocosa* or *L. nuneztovari* and the risk of CL.

4) To test for any association between the indoor abundance of the sandfly vector or CL prevalence and the control measures applied by householders.

3.2 STUDY AREA

The study was carried out within the environs of three villages: La Troja (3° 11' N, 74° 57' W), Brasilia (3° 00' N, 74° 59' W) and El Cedral (2° 56' N, 75° 00' W) belonging to the contiguous municipalities of Baraya, Tello and Neiva, respectively (Figure 3.1). The term "village" corresponds with "vereda" which is defined as a rural district in Colombia (DANE Departamento administrativo Nacional de Estadísticas 1988). In each village most of the houses are dispersed within the mountainous topography where the steepness ranges between 50 - 75%. The average altitude of the sampled houses was: 1680 m a.s.l. for La Troja; 1637 m a.s.l. for Brasilia; and 1663 m a.s.l. for El Cedral. There are spatial (rainfall) and altitudinal (rainfall and temperature) variations in climate, both within and between villages. However, the seasonal pattern of rainfall in all the three study villages follows the same bimodal pattern recorded for Huila department with two dry and two wet seasons; the first dry season is short from January to February (with the exception of the area of El Cedral where at high altitudes the season is December to January), while the second dry season is longer from July to September (Annexe 24). Total annual average rainfall showed an increasing gradient from North to South: 1082 mm in La Troja, 1338 mm in Brasilia, and 1513 mm in El Cedral, according to the records from the six nearest climatic stations in the region (IDEAM Instituto de Meteorología, Hidrología y Adecuación de Tierras 2001). This gradient in rainfall is probably related to proximity to the driest area of the department, the basin of the Cabrera River, which encloses the Tatacoa desert, located North-East of the department. Variation in rainfall with altitude seems also to occur within villages, as was observed for the two villages where rainfall data at different altitudes were available (La Troja and El Cedral), with the highest annual rainfall at the highest altitude. In La Troja the highest rainfall was 1164 mm at 2100 m a.s.l. compared with 908 mm at 1300 m a.s.l. (Annexe 24a and d). In El Cedral the highest rainfall was 1673 mm at 2100 m a.s.l. compared with 1214 mm at 1580 m a.s.l. and 1589 mm at 1100 m a.s.l. (Annexe 24b, c and f). Mean temperature, as expected, was relatively constant throughout the year, with a weak peak in September, but was significantly negatively associated with altitude. The mean temperature at 1300 m a.s.l. was 22.6°C compared with 15.3°C at 2100 m a.s.l. (Annexe 24d, e, f and a, respectively). Mean temperature estimated for the mean sampled altitude of the three villages, 1660 m a.s.l., was 18.5°C (based on the formula: $T (^{\circ}\text{C}) = 28.97 - 0.0063 h$; where h = altitude, in metres (Garcia *et al.*, 1987)). According to the Holdridge life zones, La Troja village belongs to an area

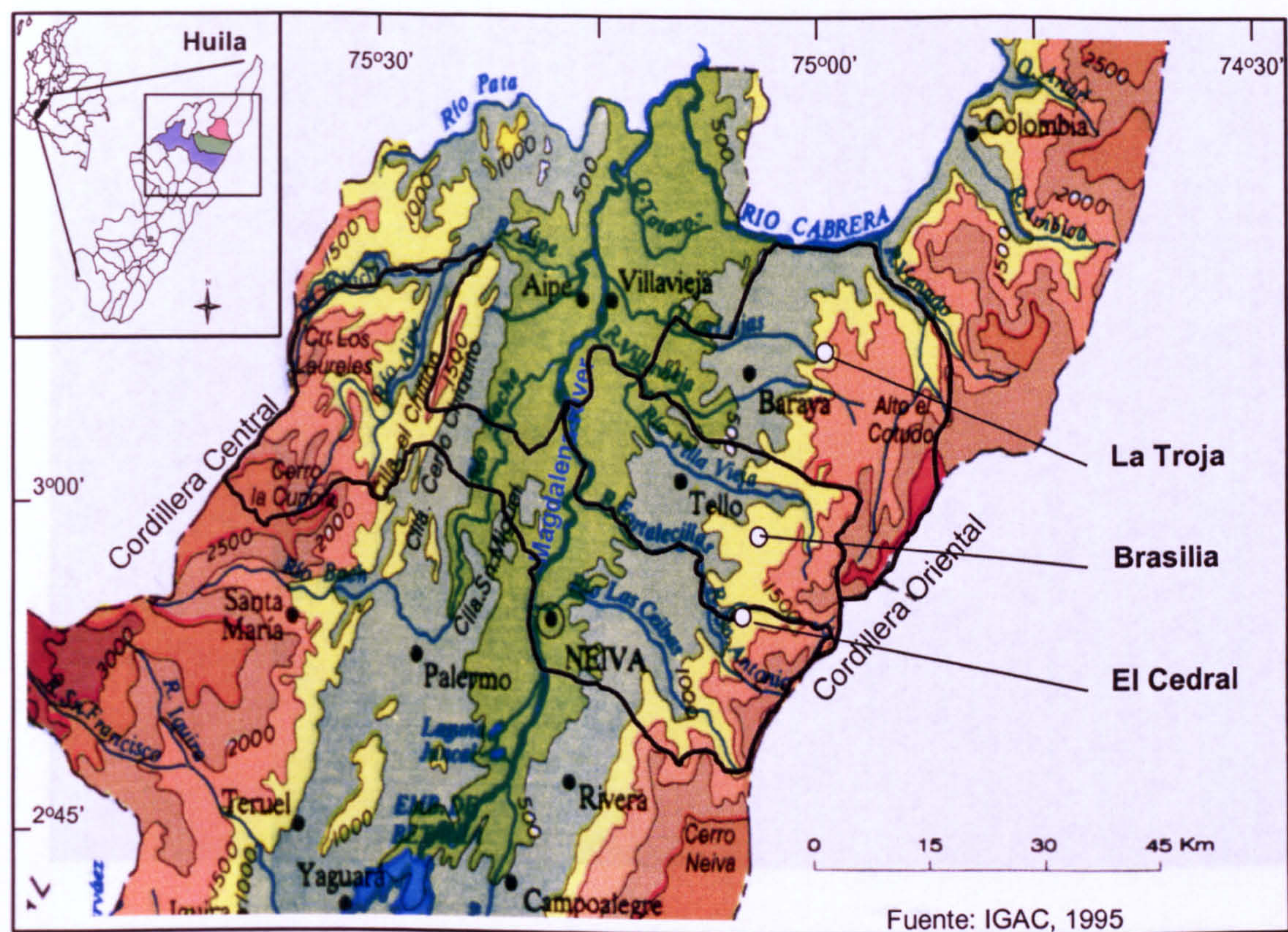


Figure 3.1 Sampled villages included in the risk factors study. Map source and colours for altitude as in Figure 1.4 .

where "bs-PM" (premontane dry forest; 500 - 1000 mm; 18 - 22°C) and "bh-PM" (premontane moist forest; 1000 - 2000 mm; 18 - 24°C) are present, while Brasilia and El Cedral are in areas with both "bh-PM" and "bmh-PM" (premontane wet forest; 2000 - 4000 mm; 18 - 24°C). The natural vegetation in the three villages has been highly disturbed and only a few remnants of the sub-Andean forest have survived in the region, except on the highest part of the mountain (above 2000 m a.s.l.) where more continuous patches of forest can be found. The soil is used mainly for pasture and coffee plantations (mainly "unshaded intensive coffee", as defined below). Other harvested crops include plantain, sugar cane, corn, yucca and some fruits (Figure 3.2). The population is formed mainly by peasants in the three villages. The number of rural inhabitants per square kilometre (inhab./km²), based on information at the municipality level, is relatively low (IGAC Instituto Geográfico Agustín Codazzi 1995). Baraya and Neiva have between 5 - 15 inhab./km², while Tello has the highest value, 15 - 25 inhab./km².

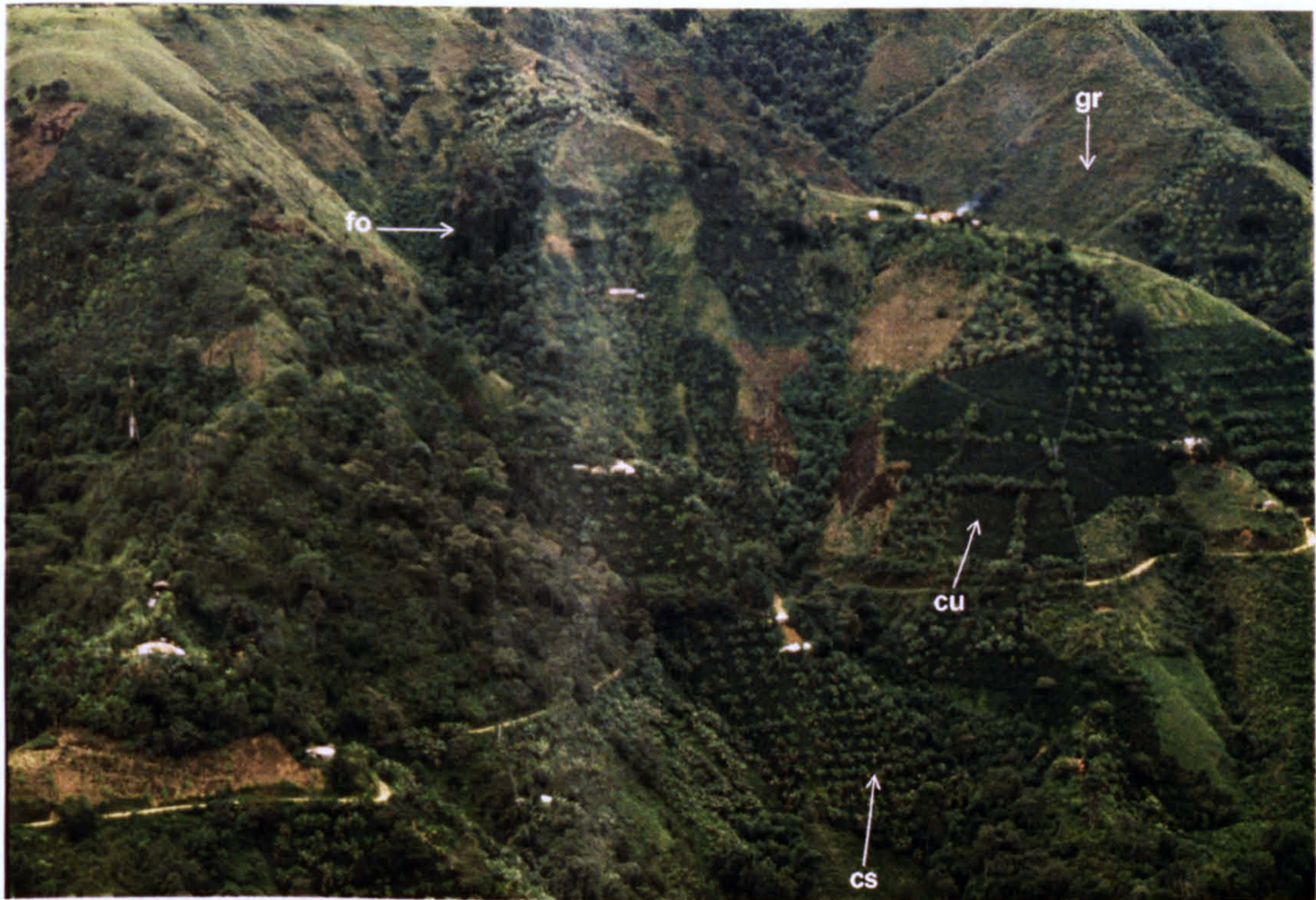


Figure 3.2 Landscape of the study area in Brasilia village (Tello municipality). cu: intensive unshaded coffee; cs: intensive semishaded coffee; fo: forest; gr: grass. Photo by Raul Pardo.

3.3 METHODS

A comparative cross sectional study was undertaken to investigate whether local variation in both indoor abundance of suspected sandfly vectors and cutaneous leishmaniasis prevalence can be explained by altitude (surrogate for climate), type of house construction, abundance of potential hosts, nature of the surrounding habitats, and/or the behaviour of householders regarding sandfly control. Information on potential risk factors and cases of cutaneous leishmaniasis was collected by questionnaires applied at household level, while sandfly abundance was recorded by sampling each house with an indoor CDC light trap.

The sample size chosen for this survey (250-300 houses) was selected from the experience of the only previous analogous study published (Quinnell and Dye 1994a). This is because to determine sample size at a given power requires a previous knowledge of the mean and variance of sandfly abundance as well as the relative frequency of the explanatory variables (which were all unknown before the survey). Quinnell & Dye (1994a), using a sample of 158 houses, identified three explanatory

variables (out of the fifteen surrounding environmental and house level variables tested) which were significantly correlated with *L. longipalpis* abundance. Hence, to be safe, in this study the target sample size was increased to 250 to 300 houses. The relatively short time between the sample collections minimised potential bias due to seasonal variation.

Three main villages were selected from three of the four municipalities within the epidemic area of Huila department, based on their high reported incidence of CL. They were: 1) La Troja, including some houses belonging to the neighbouring villages of Begonia and Totumito, in Baraya municipality; 2) Brasilia, including some houses from the neighbouring villages of Urraca Baja, Urraca Alta and Alto Roblal, in Tello municipality; and 3) El Cedral, including some houses from the neighbouring villages of El Colegio, and El Tabor in Neiva municipality; plus some houses from Barranquilla, and Puerta del Sol villages belonging to Tello municipality. All houses from the main villages were sampled. Houses from neighbouring villages were added to complete the required sample by systematic sampling up and down the altitudinal borders of the main villages. The only exclusion criterion was no sampling of houses below 1300 m a.s.l. (based on the low sandfly abundance found below this altitude during the ecological study described in Chapter 2). This survey was carried out between January to March 2001 (the season of highest abundance for sandflies).

3.3.1 Risk factors (for sandflies and CL)

A possible 34 risk factors were examined. A short description and the method of measuring each factor is given in Table 3.1. The variables included recognised, as well as possible, risk factors of the domestic environment (house features, persons per house), peridomestic environment (domestic animals, trees close to the house), and away from the house (surrounding habitats up to 300 m). For the surrounding habitats, the main emphasis focused on distinguishing the different kinds of coffee plantations since differences in *L. longiflocosa* abundance were detected between the different types of coffee plantations (Chapter 2). Types of coffee plantation were classified according to the shade given by other plants: a) “Traditional coffee”, with shade from trees moderate to heavy, b) “Intensive semishaded coffee”, with low shade by trees and other plants, and c) “Intensive unshaded coffee”, with little or not shade by trees and other plants (see Table 2.3 for a detailed description). Finally, the relative abundance of

Table 3.1 Variables which were examined as potential risk factors for sandfly abundance and prevalence of cutaneous leishmaniasis at household level.

Variable group	Name	Description	Measuring method	Tested as
Surrounding habitats features	Village	One of the three villages to which the house belong to: La Troja ^a Brasilia ^b ; and El Cedraí ^c .		Categorical
	Altitude	Altitude (m asl) where each house was located. Analysis based on eight ranges: 1300-1399, 1400-1499, 1500-1599, 1600- 1699, 1700-1799, 1800-1899, 1900-1999, 2000-2200.	Altimeter	Categorical
	Vegetation within 300 m	Type of vegetation (forest, traditional coffee, intensive semishaded and unshaded coffee, grass, sugar cane and banana plants).	Estimated by direct observation	Continuous
	Vegetation within 50 m	Number of trees 2 - 10 m high	Direct counting	Discrete
		Number of trees >10 m high	Direct counting	Discrete
		Number of banana plants	Categorized by direct observation	Categorical
		Percentage of cover	Estimated by direct observation	Continuous
	Distance to the nearest house		Estimated by direct observation	Continuous
	Number of houses within 100 m		Direct counting	Discrete
	Animal shelters (within 200 m)	Presence of animal shelters.	Direct observation	Binary
House features	Housing type	Type of house (house or hut). Hut was defined as a construction of poor quality, usually with one bedroom, floor of soil-earth and walls with cracks and with large openings.	Categorized by direct observation	Categorical

Table 3.1 continued.

Variable group	Name	Description	Measuring method	Tested as
	Wall type	Type of wall according to four categories based on the materials used in the construction: ("bahareque", brick, wood, stone and cement). "Bahareque" was defined as a type of wall made of a mix of mud and cow manure with a internal framework of bamboo.	Categorized by direct observation	Categorical
	Wall cracks	Cracks on the walls according to four categories of percentage of affected area: 0%, 1-30%, 31-60%, >60%.	Categorized by direct observation	Categorical
	Smooth walls	Wall smooth or not.	Categorized by direct observation	Binary
	Ceiling type	Type of ceiling according to four categories: close plank, close plank and hole (to get access into the ceiling), plank with spaces and no ceiling.	Categorized by direct observation	Categorical
	Total openings	Total external openings (m ²) in the house.	Tape measure	Continuous
	Time with electricity service	Time (years) since electricity service was installed in the house.	Informed by the interviewee	Continuous
Number of potential hosts	Persons per house	Number of pers itly in the house.	Informed by the interviewee	Discrete
	Domestic animals (dogs, chickens, cows, equines, pigs, cats)	Number of domestic animals within 200m of the house.	Informed by the interviewee	Discrete
Vector control measures	Number of bednets per house	Number of bednets found in the house.	Direct counting	Discrete
	House spraying with insecticides	Use or not of spraying to control sandflies since the family moved to the house.	Informed by the interviewee	Binary
	House spraying with non-insecticidal substances	Use of spraying with non-insecticidal substances to control sandflies since the family moved to the house.	Informed by the interviewee	Binary
	Smoke	Use or not of smoke (rising from a small fire made of different materials) to repel sandflies since the family moved to the house.	Informed by the interviewee	Binary

^a Including some houses from Begonia and Totumito villages; ^b Including some houses from Alta Urraca and Alto Roblai; ^c Including some houses from El Colegio and El Tabor, Barranquilla, and Puerta del Sol (the last two belonging to the neighbouring municipality of Tello).

banana plants (within 50 m of the house) was included as a possible risk factor, based on the significant negative association detected by univariate analysis between this plant and the abundance of *L. longiflocosa*, also during the ecological study.

3.3.1.1 Data collection

Two questionnaires were created to collect the field data. The first questionnaire described general information (place, altitude, length of residence), demographic information (sex, age), and the potential risk factors (house and environmental features) (Annexe 25). Additional information on behaviour regarding control measures for sandflies or CL, by the householders, was also included in the questionnaire (which will be covered in detail later in Chapter 5) as they could have an effect on sandfly indoor activity.

The second questionnaire included detailed information on each suspected CL case. It included information on place of residence when he or she got the disease, age, type of diagnosis, and treatment (Annexe 26).

The questionnaires were piloted in June 2000 within the study area and some adjustments were implemented accordingly. Two interviewers, the head of the project (the candidate) and a trained entomologist from the SSDH, conducted personal interviews in the selected houses. These questionnaires were answered by the head of the household or his partner for the questions on general information, demography (family members, age and sex) and common risk factors for household members. Details regarding house construction and environmental risk factors around the house were recorded by the interviewers by observation or by measurement, with the assistance of two health workers from the relevant municipality. When more than one family lived in the house, separate questionnaires were filled in for each one. Movement of families within the study area was also recorded and the length of residence in the former house was taken into account in the final analysis.

3.3.1.2 Data Management

Initially two main databases were created, the first included mainly the risk factor data and the second the demographic and CL case data. These databases were digitalized using Epi-Info software, Version 6.04d (CDC 2001). The risk factor database was merged with a third database, the sandfly data (created in Excel), in order to generate a fourth database for analysing the sandfly risk factors. Finally, this data base was merged with the demographic and CL database to provide a fifth database on which the analysis of CL risk factors and the association between CL prevalence and sandflies was performed (Figure 3.3).

To ensure data quality and to reduce possible collecting errors (response errors) and processing errors, several measures were taking during the data collection and processing (Figure 3.3). Manual checking of the completed questionnaire was carried out during the field work. Interactive checking was performed (key unique, range, legal values and conditional jumps) during the data entry in London using the check module of Epi-Info. Checking for consistency was performed on each generated database. The risk factor database was initially validated by dual entry of 10% of the data by two persons. The complete database was then checked for those parameters where errors had been detected.

Validation of CL cases was carried out by comparing the cases detected during the trial with the epidemiological records of CL patients held by the SSDH. Missing data were excluded from the analysis. Erroneous data were recorded as missing data.

3.3.2 Sandfly sampling

In addition to the questionnaire survey, each house was sampled with a CDC light trap (Sudia and Chamberlain 1962) on one night (18:00 to 7:00 h) in order to measure the abundance of sandflies for testing against the risk factor variables. The CDC light trap was set up in one inhabited bedroom at a height of 1.5 m, close to the foot end of a bed. Inhabitants were requested not to apply any control measures during the sampling night, or if it was found to be necessary because of sandfly nuisance, they were instructed to inform a member of the field team when he picked up the trap the following morning. In rare cases when heavy rain (for longer than five hours) took place during the sampling,

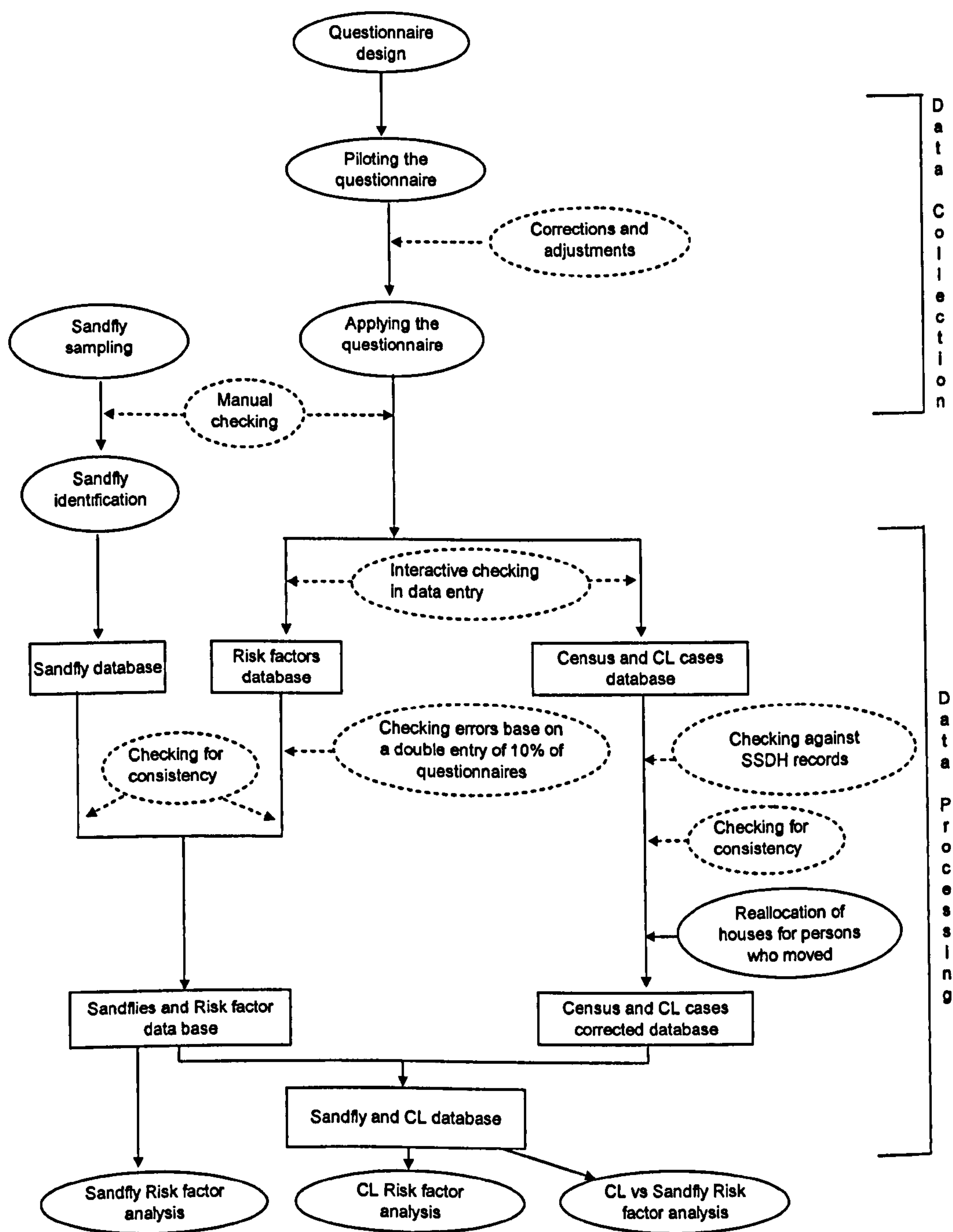


Figure 3.3 Diagram of the general data management for the risk factor study. SSDH: Huila Health Service; CL: Cutaneous leishmaniasis. Rectangular boxes are databases, elliptical boxes with continuous lines enclosed activities, and elliptical boxes with dashed lines enclosed checking and validation of data.

the CDC light trap was set up for an additional night and the first sample was omitted from the study. Collected sandflies were separated in a field station from other insects, killed with chloroform, counted by sex, and preserved in vials with 70% ethanol. For each sample a form (similar to that shows in Annexe 2) was completed including a basic

field description. Identification was carried out at the Laboratory of Entomology from the INS in Bogotá. Sandflies were exposed for a few minutes to a hot 10% KOH solution, before clearing in phenol solution, and examining under a phase contrast microscope. Identification was carried out using the keys of Young (1979) and Young and Duncan (1994). Specimens were compared with the reference collection for Huila department produced during the ecological study described in Chapter 2.

In addition, to help in the design of the Intervention Trial in Chapter 4, the number of blood-fed females captured was recorded, including blood condition (fresh, bright red or digested, dark brown or black) and amount (percentage of abdomen with blood: low, 25% or less; half 50%; full, $\geq 75\%$). These fed females were kept separate, the majority in 70% ethanol and in some the blood was squashed onto filter paper (Whatman No. 2). The paper samples were kept dry by storing them in plastic bags containing desiccant until they were analysed in the laboratory. The thorax and remaining abdomen of each fed female were stored individually in the same way as males and unfed females.

A database was generated in Excel and merged with the risk factors and the demographic and cases databases in order to carry out the required analyses (Figure 3.3).

3.3.3 CL Cases

Cured and active cases of CL were included in the study as well as MCL cases. CL cases were recorded as confirmed if they matched all of the following criteria:

- 1) The patient said they had been diagnosed (clinically or parasitologically) as CL by a member of the SSDH (doctor or nurse).
- 2) The patient had received the appropriate CL treatment of glucantime during a period of at least 20 days (Ministerio de Salud de Colombia 1994).
- 3) The patient reported being cured as a result of the treatment.

Criterion number 2 was not applied to women who got CL while they were pregnant. Also, potential MCL cases were recorded as former confirmed CL cases if they fulfilled

the three criteria described above. In addition, the presence of scars was checked if the patients were present.

Persons found with lesions compatible with CL (possible new case, reactivation or reinfection) or suspicious of MCL, were sent to the nearest Health Service of their municipality (who agreed to take part in the study) where diagnosis and treatment were expected to be given. In order to follow these cases a form was given to the patient and copies were delivered to the respective Municipality Health Service and the SSDH. The co-ordinator (the candidate) of the study was in charge of monitoring the evolution of the cases (Annexe 27).

Cases were included in the study if the person acquired the disease while resident in their current house or in another house included in the survey. For the latter situation, for the risk factor analysis the patient was recorded in the former house and dropped from the records of the current house. The outcome measurement of the risk factor analysis was the number of cases in a household divided by the total number of householders. Householders were only included in the analysis if they had inhabited one of the survey houses for at least three months per year.

Cases were excluded from the study if: 1) a person had got the disease in the study area but, had left the area before the questionnaire was applied; 2) there was strongly suggestive evidence that the person had caught the disease outside the study area.

3.3.4 Statistical analysis

The analysis focused on the only two species present in reasonable numbers: *L. longiflocosa* and *L. nuneztovari*: 93.5% and 2.1%, respectively, of all sandflies collected. Because of the high overdispersion of the sandfly data, catches are presented as Williams' geometric means (GM), including their 95% confidence intervals, except where otherwise stated. As zeros were present in the original data set the log transformation used was: $\ln(x + 1)$. After testing for the distribution of the sandfly data, it was found that the log transformed data for *L. longiflocosa* agreed with the Poisson distribution, using the normal approximation to the χ^2 value (used for large samples, $n > 101$) (Krebs 1999). Nevertheless, the assumption of normal errors was used in the

final analysis of the log transformed data, as the error distribution for the Minimum Adequate Model (MAM) did not differ significantly from the normal distribution. The analysis was also carried out assuming Poisson errors (not shown), and the results were similar to those described in this chapter. In contrast, the *L. nuneztovari* raw data were assumed to follow a negative binomial distribution for the analysis. These data were well described by this distribution, after applying maximum likelihood to estimate the negative binomial exponent (k) and the U statistic goodness of fit test (Krebs 1999) using the Programs for Ecological Methodology software (Krebs 2002).

Multivariate statistical analysis was generally applied using the Generalized Linear Models approach (GLIM) (Crawley 1993) and Stata 7 software (Stata Corporation 2001). Initially, a maximum model including all possible explanatory variables (risk factors) was generated. Then, the model was simplified by backward elimination of the least significant explanatory variables, using for this purpose the Wald test or the likelihood ratio test (LRT), whichever was appropriate. Finally, a Minimum Adequate Model (MAM) was obtained where all the explanatory variables were significant ($p < 0.05$). The leverage of points method was performed for detecting outlier points applying the formula provided by Crawley* (Crawley 1993), removing these values from the model and checking for changes in the significance of the explanatory variables. The points were retained in the model if no change was observed. Validation of the MAM model was carried out by checking the appropriate residuals plots and Quantile-Quantile plots.

Logistic regression analysis, controlling by total time of residence in each house, time spent per year in each house, age, sex and house was used to test for CL risk factors and to test for the relationship between the log transformed abundance of *L. longiflocosa* or *L. nuneztovari* and CL prevalence. In both analyses the outcome measurement was binary: CL presence (1) or absence (0).

In order to illustrate the analyses, graphics for the two species of interest (*L. longiflocosa* and *L. nuneztovari*) are presented on the same page. Note that statistical differences cannot always be deduced from these graphics, as the analyses were multivariate. In addition in some of the graphics continuous variables have been transformed to categorical values (i.e. ranges) for illustrative purposes.

* $h_i = \frac{1}{n} + \frac{(x_i - \bar{x})^2}{\sum (x_i - \bar{x})^2}$ A data point was considered an outlier point if $h_i > \frac{2p}{2}$ where p is the number of parameters in the model.

3.3.5 Ethical considerations

Meetings, at village level, with the community in the three sampled municipalities were arranged to explain the aims, methods and usefulness of the study in order to get their acceptance and collaboration. Confidentiality of the collected data was guaranteed. Also it was made clear that the collected data would be used only for the purposes of the study. The same procedure was completed when each house was visited. It is considered, by the MOH of Colombia, that there is no risk to a population as a result of an intervention using questionnaires (resolution No. 008430 1993).

3.4 RESULTS

A description of the 34 potential risk factors tested is presented in Annexe 28. A total of 271 houses were included in the survey distributed roughly equally between the three study villages: Brasilia, 36% (98 / 271); La Troja, 32%, (87 / 271), and El Cedral, 32%, (86 / 271). The average altitude for the sampled houses was 1655 m. Houses were dispersed, with an average distance between nearest houses of 147 m.

Most of the buildings were classified as houses, 83.4% (226 / 271), rather than "huts". Walls were made mainly of "bahareque", 58.4% (156 / 267), followed by brick, 19.1% (51 / 267), and wood, 16.9% (45 / 267). Most of the walls were in relatively good condition with no cracks, 56% (149 / 265), or very few, 24.5% (65 / 265). All sampled houses had roofs made of zinc, mostly without a ceiling, 35.8% (93 / 260), or with a ceiling made of planks either with [24.2% (63 / 260)] or without [6.9% (70 / 260)] spaces between the planks. Most of the houses presented relatively large openings for sandflies to enter - with an average total opening area of 5.8 m². Most houses had an electricity service, 89.5% (231 / 258), for an average of 9.1 years.

Houses were inhabited by a mean of 5.4 persons who had lived there for an average time of 10.2 years, each person spending an average of 10.5 months/year in the house. The percentage of houses with each kind of domestic animal and the average number per house (for the "positive" houses), within a radius of 200 m, were as follows: chickens, 90.7% (13.4 chickens/house); dogs, 73.8% (2.0 dogs/house); pigs, 23.4% (2.0 pigs/house); equines, 22.2% (1.9 equines/house); cats, 19.2% (1.3 cats/house) and cows, 13% (7.3 cows/house). Domestic animals lived mainly in the peridomicile or further,

with 59.8% (162 / 271) of houses providing some kind of shelter located in the peridomestic area. Other domestic and wild animals which were found in very low numbers were not included in the analysis.

With respect to wild animals, it is important to note that households frequently reported the presence, within 300 m radius from the houses, of a relatively large variety of species. The most commonly named, with their scientific name (Rodriguez *et al.*, 1995), grouped by order were:

DIDELPHIMORPHA

Opposum "Chucha" *Didelphis marsupialis* Linnaeus, 1758

CINGULATA

Armadillo "Armadillo" *Dasypus novemcinctus* Linnaeus, 1758

CARNIVORA

Fox "Zorro" *Cerdocyon thous* (Linnaeus, 1766)

Tayra "Ulama" *Eira barbara* (Linnaeus, 1758)

Kinkajou "Perro de monte" *Potos flavus* (Schreber, 1774)

Coati "Cusumbo" *Nasua nasua* (Linnaeus, 1766)

Weasel "Comadreja" *Mustela frenata* Liechtenstein, 1831

Jaguarundi "Gato de monte" *Herpailurus yagouaroundi* (E. Geoffroy St. Hilaire, 1803)

RODENTIA

Squirrel "Ardilla" *Sciurus granatensis* Humboldt, 1811

Porcupine "Puerco espin" *Coendou prehensilis* (Linnaeus, 1758)

Agouti "Guara" *Dasyprocta fuliginosa* Wagler, 1832

Agouti "Guatin" *Dasyprocta punctata* Gray, 1842

Paca "Boruga" *Agouti paca* (Linnaeus, 1766)

LAGOMORPHA

Mountain rabbit "Conejo de monte" *Sylvilagus brasiliensis* (Linnaeus, 1758)

EDENTATA

Sloth "Perezoso" *Choleopus sp.*

Ant-eater "Oso hormiguero" *Tamandua sp.*

As wild animals very probably play a role as reservoirs of CL, these data should be taken in to account in future studies.

In the peridomestic environment, within a 50 m radius, houses were surrounded on average by 24.3 trees between 2 - 10 m high and 6.7 trees higher than 10 m, with 82.7% (224 / 271) of houses surrounded by more than 10 banana plants. Although the number of trees around the houses was relatively high the mean percentage of cover, by trees higher than 2 m, was only 13.9%. The percentage of houses surrounded by each type of vegetation (within 300 m radius) and the average percent of land covered with that vegetation type (around "positive" houses) was as follows: grass: present around 94.8% (257 / 271) of houses, with an average percent cover of 45.0%; forest: around 90.4% (245 / 271) of houses, with average cover of 14.1%; intensive unshaded coffee: around 61.6% (167 / 271) of houses, average cover of 47.9%; intensive semi-shaded coffee: around 24.7% (67 / 271) of houses, average cover of 31.6%; banana: around 19.2% (52 / 271) of houses, average cover of 11.5%; sugar cane: around 17.7% (48 / 271) of houses, average cover of 8.1%; and traditional coffee: around 12.6% (34 / 271) of houses, average cover of 24.5%. Other types of vegetation (corn and fruit crops) were found around less than 6% of houses and were excluded from the analysis.

The most common control measure for sandflies used by the householders was smoke, practiced by 52.2% (140 / 268) of householders. The second most common was the use of bednets, by 30.1% (81 / 269) of householders, with an average of 1.8 bednets/house (positive houses for bednets only). The third most common measure was house spraying with insecticides (domestic or agricultural), by 26.1% (70 / 268) of householders. The least common control measure was spraying with a non-insecticidal substance (such as gasoline, kerosene, and creolin), by 18.7% (50 / 268) of householders.

3.4.1 Risk factor for sandfly vectors at household level

A total of 7,659 sandflies belonging to at least 8 species were caught inside 265 houses from a total of 271 sampled during the survey (Table 3.2); the remaining 6 houses were

Table 3.2 Composition and relative abundance of sandflies caught with CDC light traps inside houses of the three sampled villages during the risk factors survey.

Lutzomyia species	Village											
	La Troja (Baraya municipality) ^a (n = 87)				Brasilia (Tello municipality) ^b (n = 93)				El Cedral (Neiva municipality) ^c (n = 85)			
	♀ (%)	♂ (%)	Total (%)	♀ (%)	♂ (%)	Total (%)	♀ (%)	♂ (%)	♀ (%)	♂ (%)	Total (%)	Total (n = 265)
<i>L. longiflocosa</i>	4088 (96.4)	493 (90.6)	4581 (93.6)	968 (97.3)	122 (87.8)	1090 (93.3)	1446 (96.7)	45 (83.3)	1491 (93.5)	6502 (96.6)	660 (89.6)	7162 (93.5)
<i>L. nuneztovari</i>	87 (2.05)	13 (2.39)	100 (2.04)	17 (1.70)	6 (4.31)	23 (1.96)	34 (2.27)	6 (11.1)	40 (2.51)	138 (2.04)	25 (3.39)	163 (2.13)
<i>L. trinidadensis</i>	53 (1.25)	35 (6.43)	88 (1.80)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	53 (0.79)	35 (4.75)	88 (1.15)
<i>L. (Helcocyrtomyia) spp.</i>	2 (0.05)	0 (0)	2 (0.04)	4 (0.40)	9 (6.47)	13 (1.11)	14 (0.94)	2 (3.70)	16 (1.00)	20 (0.30)	11 (1.49)	31 (0.40)
<i>L. columbiana</i>	9 (0.21)	0 (0)	9 (0.18)	6 (0.60)	1 (0.72)	7 (0.60)	2 (0.13)	0 (0)	2 (0.13)	17 (0.25)	1 (0.14)	18 (0.23)
<i>L. dubitans</i>	1 (0.02)	2 (0.37)	3 (0.06)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.01)	2 (0.27)	3 (0.04)
<i>L. oresbia</i>	1 (0.02)	1 (0.18)	2 (0.04)	0 (0)	1 (0.72)	1 (0.09)	0 (0)	0 (0)	0 (0)	1 (0.01)	2 (0.27)	3 (0.04)
<i>L. erwindonaldi</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.85)	1 (0.06)	0 (0)	1 (0.14)	1 (0.01)
Unidentified			112 (2.29)			34 (2.91)			44 (2.76)			190 (2.48)
Total	4241	544	4897	995	139	1168	1496	54	1594	6732	737	7659

^a Including some houses from Begonia and Totumito Villages; ^b Including some houses from Alta Urraca, Baja Urraca and Alto Roblal; ^c Including some houses from El Colegio and El Tabor, Barranquilla, and Puerta del Sol (the last two belonging to the neighboring municipality of Tello).

excluded because the CDC traps did not work properly (3 samples) or because the householders altered the conditions of the catches (3 samples). *Lutzomyia longiflocosa* was easily the most dominant species, accounting for 93.5% (7,162 / 7,659) of all captures. The second most common species was *L. nuneztovari* accounting for 2.1% (163 / 7,659). The other six species represented very low percentages of the total captured: *L. trinidadensis*, 1.2% (88 / 7,659), *L. columbiana*, 0.23% (18 / 7,659), *L. dubitans*, 0.04% (3 / 7,659), *L. oresbia*, 0.04% (3 / 7,659), *L. erwindonaldi*, 0.01% (1 / 7,659), and an unidentified species (probably two or three species) of the *Helcocyrtomyia* subgenus, 0.40% (31 / 7,659). The remaining 2.5% (190 / 7,659) were sandflies not identifiable because of damage during manipulation (most of them presumably *L. longiflocosa*).

A similar pattern of very high dominance of *L. longiflocosa* with a minority of *L. nuneztovari* and few of the other species was observed in each of the three sampled villages (La Troja, Brasilia, and El Cedral). This similarity in the sandfly community was confirmed by the Morisita index of similarity, which ranges from 0 (no similar) to 1 (completely similar) (Krebs 1999). The index was 0.984 between Brasilia and El Cedral villages; 0.875 between Brasilia and La Troja; and 0.858 between La Troja and El Cedral.

Nevertheless, there was a significant inter-village difference in the mean abundance of the two main sandfly species, *L. longiflocosa* (ANOVA, $F_{(2, 264)} = 6.04$, $p = 0.0027$) and *L. nuneztovari* (Negative binomial regression, $X^2_{(2)} = 19.39$, $p = 0.0001$) between villages. *L. longiflocosa* had a significantly higher abundance in La Troja village, GM = 9.9 (6.7 - 14) sandflies/CDC light trap/night (s/LT/n), compared with Brasilia, GM = 4.4 (3.1 - 6.0) s/LT/n ($z = -3.34$, $p = 0.001$), and El Cedral, GM = 5.4 (3.9 - 7.3) s/LT/n ($z = -2.50$, $p = 0.012$) (Annexe 29). No statistical difference in *L. longiflocosa* abundance was detected between Brasilia and El Cedral municipalities ($z = 0.78$, $p = 0.435$). The same pattern was observed with *L. nuneztovari*, with significantly higher abundance in La Troja, GM = 0.58 (0.37 - 0.82) s/LT/n, compared with Brasilia, GM = 0.15 (0.07 - 0.24) s/LT/n ($z = -4.36$, $p < 0.001$), and El Cedral, GM = 0.28 (0.15 - 0.41) s/LT/n ($z = -2.71$, $p = 0.007$) (Annexe 30). Although the abundance of *L. nuneztovari* in El Cedral was almost twice that in Brasilia, this difference was not significant ($z = 1.72$, $p = 0.086$).

The distribution of the raw data of the indoor CDC light traps captures of the two main sandfly species (Annexes 31 and 32) describes, as expected, an aggregated (clumped) pattern – especially for *L. longiflocosa*, whose index of dispersion (variance/mean ratio) was considerably higher, 420 (11,343 / 27), than that of *L. nuneztovari*, 4.4 (2.67 / 0.61). It should be noted that 80.3% (5,751 / 7,162) of the total number of *L. longiflocosa* were caught in 15.8% (42 / 265) of the houses; for this group of houses the GM number of this species was 76 (57 - 101) s/LT/n, i.e. twelve times higher than the GM of *L. longiflocosa* for all sampled houses, 6.2 (5.0 - 7.5) s/LT/n. A similar situation was found for *L. nuneztovari* where 80.4% (131 / 163) of the total number of sandflies were caught in 14.7% (39 / 265) of the houses; and for this group the GM number of this species was 2.7 (2.2 - 3.4), s/LT/n, i.e. 9 times higher than the GM of *L. nuneztovari* for all houses, 0.3 (0.2 - 0.4).

In spite of the aggregated distribution of the catches, some sandflies were found inside a remarkably high percentage of houses: 89.1% (236 / 265) (Table 3.3). *L. longiflocosa* was found in 85.7% (227 / 265) of all houses, and *L. nuneztovari* in 26.8% (71 / 265). Species less frequently found were *L. columbiana*, 6% (16 / 265), and *L. (Helcocyrtomyia) spp.* with 7.2% (19 / 265).

Finally, the sex ratio (male:female) was strongly female biased for the two main species, 1:9.9 for *L. longiflocosa* and 1:5.5 for *L. nuneztovari*. As transmission is by the bite of female sandflies, individual analysis for the males is not presented.

Considering the overwhelming dominance of *L. longiflocosa* and the relatively high frequency of *L. nuneztovari* among the remaining sandflies, the analysis on sandfly risk factors was carried out on these two species only. For comparative purposes the figures included in the results will show the data for both species on the same page.

Table 3.3 Percentage of positive houses for the epidemiologically most important sandfly species caught with indoor CDC light traps during the risk factor survey.

Lutzomyia species	Village							
	La Troja (n=87)		Brasilia (n=93)		El Cedral (n=85)		Total (n=265)	
	No. houses +	%	No. houses +	%	No. houses +	%	No. houses +	%
<i>L. longiflocosa</i>	77	88,5	73	78,5	76	89,4	227	85,7
<i>L. nuneztovari</i>	34	39,1	15	16,1	22	25,9	71	26,8
<i>L. columbiana</i>	8	9,2	6	6,5	2	2,4	16	6,0
<i>L. (Helcocyrtomyia) spp.</i>	2	2,3	7	7,5	10	11,8	19	7,2
All species	82	94,3	77	82,8	77	90,6	236	89,1

3.4.1.1 Risk factors for *Lutzomyia longiflocosa*

Stepwise elimination from the maximal model incorporating the 34 potential risk factors for indoor *L. longiflocosa* abundance (ln [x + 1]) led to a Minimum Adequate Model (MAM) with six variables (Table 3.4): village; altitude; percentage of land covered by grass within 300 m of the house; number of houses within 100 m; number of persons per house; and number of dogs within 200 m. The MAM explained 26.3% of the household variance (as measured by r^2) in *L. longiflocosa* indoor abundance (Annexe 33). The variables with the most explanatory power were village (which explained 8.9% of the variance) and altitude (6.4%). The explanatory power of the remaining variables was, in decreasing order: number of dogs, 4.6%; number of persons per house, 3.8%; number of houses within 100 m, 3.1%; and percentage of grass, 2.8%.

The goodness of fit of the MAM is illustrated by three graphs: 1) the plots of raw residuals against the fitted values (Annexe 34) where the variance seems relatively constant with the increase in the predicted abundance of *L. longiflocosa*; 2) the plot of the predicted against the observed abundance (Annexe 35) where it is clear that the model agrees with the observed data; and 3) the plot of the quantiles of residuals against the quantiles of the normal distribution (Q-Q plot) where the normal distribution of the residuals was confirmed (Annexe 36).

Table 3.4 Risk factors for indoor *Lutzomyia longiflocosa* abundance (log transformed number/CDC trap/house-night) identified by multivariate analysis.

Explanatory variable		Coefficient	S.E.	z	P>z	95% C.I.	
						Min	Max
Village	La Troja ^a						
	Brasilia	-1.147	0.209	-5.48	<0.001	-1.557	-0.737
	El Cedral	-0.489	0.227	-2.15	0.031	-0.935	-0.043
Altitude	1600 - 1699 ^a						
	1300 - 1399	-1.423	0.551	-2.58	0.010	-2.503	-0.343
	1400 - 1499	-0.516	0.300	-1.72	0.086	-1.104	0.073
	1500 - 1599	-0.485	0.222	-2.18	0.029	-0.920	-0.049
	1700 - 1799	-0.386	0.249	-1.55	0.122	-0.874	0.103
	1800 - 1899	-0.809	0.327	-2.47	0.013	-1.451	-0.167
	1900 - 1999	-1.310	0.475	-2.76	0.006	-2.241	-0.379
	2000 - 2200	-1.708	0.540	-3.16	0.002	-2.767	-0.649
Surrounding habitats features							
Percentage of grass (within 300 m)		-0.010	0.003	-3.07	0.002	-0.017	-0.004
Number of houses (within 100 m)		-0.167	0.052	-3.25	0.001	-0.269	-0.066
Potential hosts							
Number of dogs (within 200 m)		-0.251	0.064	-3.95	<0.001	-0.376	-0.126
Number of persons per house		0.108	0.030	3.57	<0.001	0.049	0.168
Intercept		1.988	0.604	3.29	0.001	0.804	3.172

^aBaseline Category.

Comparing villages, the mean abundance of *L. longiflocosa* was significantly higher in La Troja, 9.9 s/LT/n, then in either Brasilia, 4.4 s/LT/n ($z = -5.48, p < 0.001$), or El Cedral, 5.4 ($z = -2.15, p = 0.031$). Surprisingly, after adjusting for the other 5 variables in the MAM, the mean abundance of *L. longiflocosa* in El Cedral was also significantly higher (in the MAM) than in Brasilia ($z = -2.93, p = 0.031$) (Annexe 29).

For altitude, *L. longiflocosa* showed the highest mean indoor abundance, 11 (6.8 - 7) s/LT/n, in the range 1600 - 1699 m a.s.l. (Figure 3.4), which was significantly greater than the mean abundance in most other ranges: 1.6 s/LT/n at 1300 - 1399 m a.s.l.; 5.5 s/LT/n at 1500 - 1599 m a.s.l.; 5.5 s/LT/n at 1800 - 1899; 1.4 s/LT/n at 1900 - 1999 m a.s.l.; and 0.4 at 2000 - 2200 m a.s.l. The mean abundance between 1600 - 1699 was also greater (though not significantly so) than the mean abundance detected within the ranges 1400 - 1499 m a.s.l. (6.8 s/LT/n) and 1700 - 1799 m a.s.l. (also 6.8 s/LT/n). The effect of extreme altitudes, 1300 - 1399 m a.s.l. and 2000 - 2200 m a.s.l., compared with the abundance of *L. longiflocosa* in the range 1600 - 1699 m a.s.l. should be noted. In these two ranges the mean abundance of *L. longiflocosa* was reduced by 76% ($[e^{-1.423}] - 1$) and 82% ($[e^{-1.708}] - 1$), respectively.

Regarding the impact of surrounding habitats, the percentage of land covered with grass within 300 m of each house had a significantly negative relationship with the abundance of *L. longiflocosa* inside the house: for each increase of 10% in grass cover around a house the mean number of indoor *L. longiflocosa* females/LT/n decreased by 9.5% ($[e^{-0.01 \times 10}] - 1$) (Figure 3.6). The indoor abundance of *L. longiflocosa* also decreased significantly with the number of houses within 100 m: for each increase in one surrounding house the mean number of *L. longiflocosa* females/LT/n decreased by 15% ($[e^{-0.167}] - 1$) (Figure 3.8).

Regarding the impact of potential bloodmeal sources, a significant negative relationship was detected between the number of dogs within 200 m from house and the indoor abundance of *L. longiflocosa*: for each increase in one dog/house the mean number of indoor *L. longiflocosa* females/LT/n decreased by 22% ($[e^{-0.251}] - 1$) (Figure 3.10). In contrast, the number of persons per house was positively associated with the indoor abundance of *L. longiflocosa*: for each increase in one person/house the mean number of indoor *L. longiflocosa* females/LT/n increased by 11% ($[e^{0.108}] - 1$) (Figure 3.12).

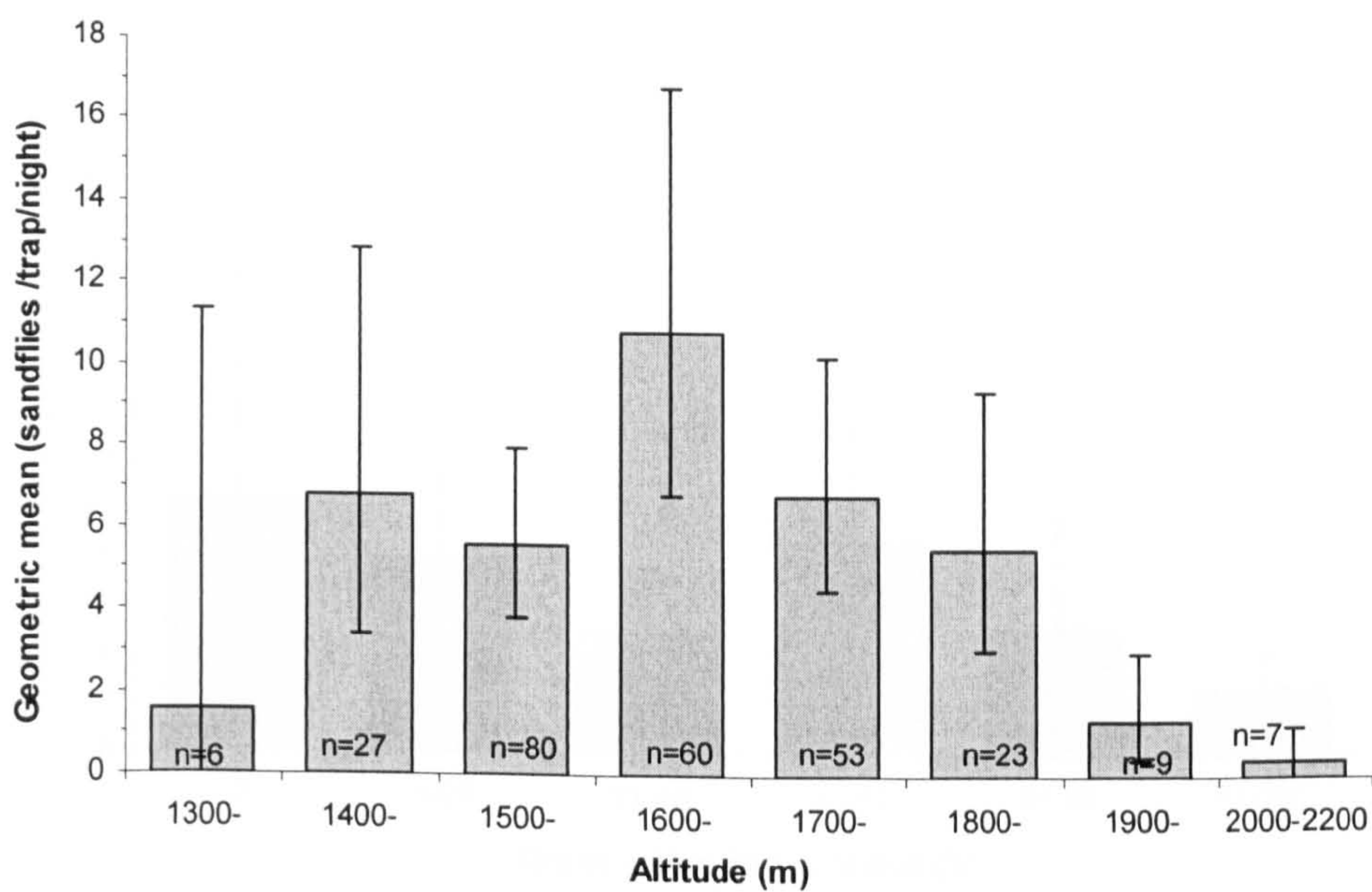


Figure 3.4 The relationship between altitude and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

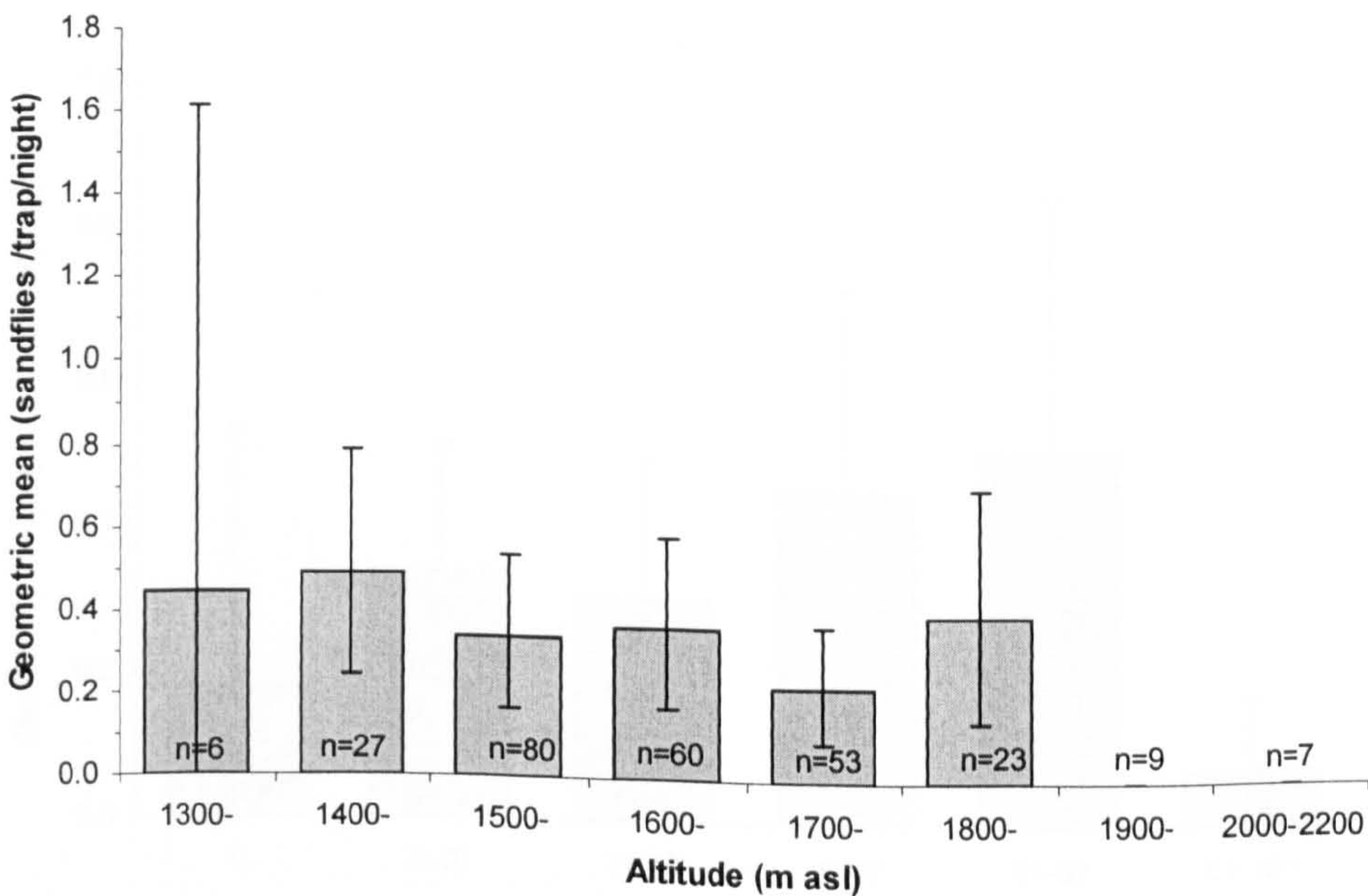


Figure 3.5 The relationship between altitude and *Lutzomyia nuneztovari* abundance (as measured by CDC light traps). Error bars as in Figure 3.4 .

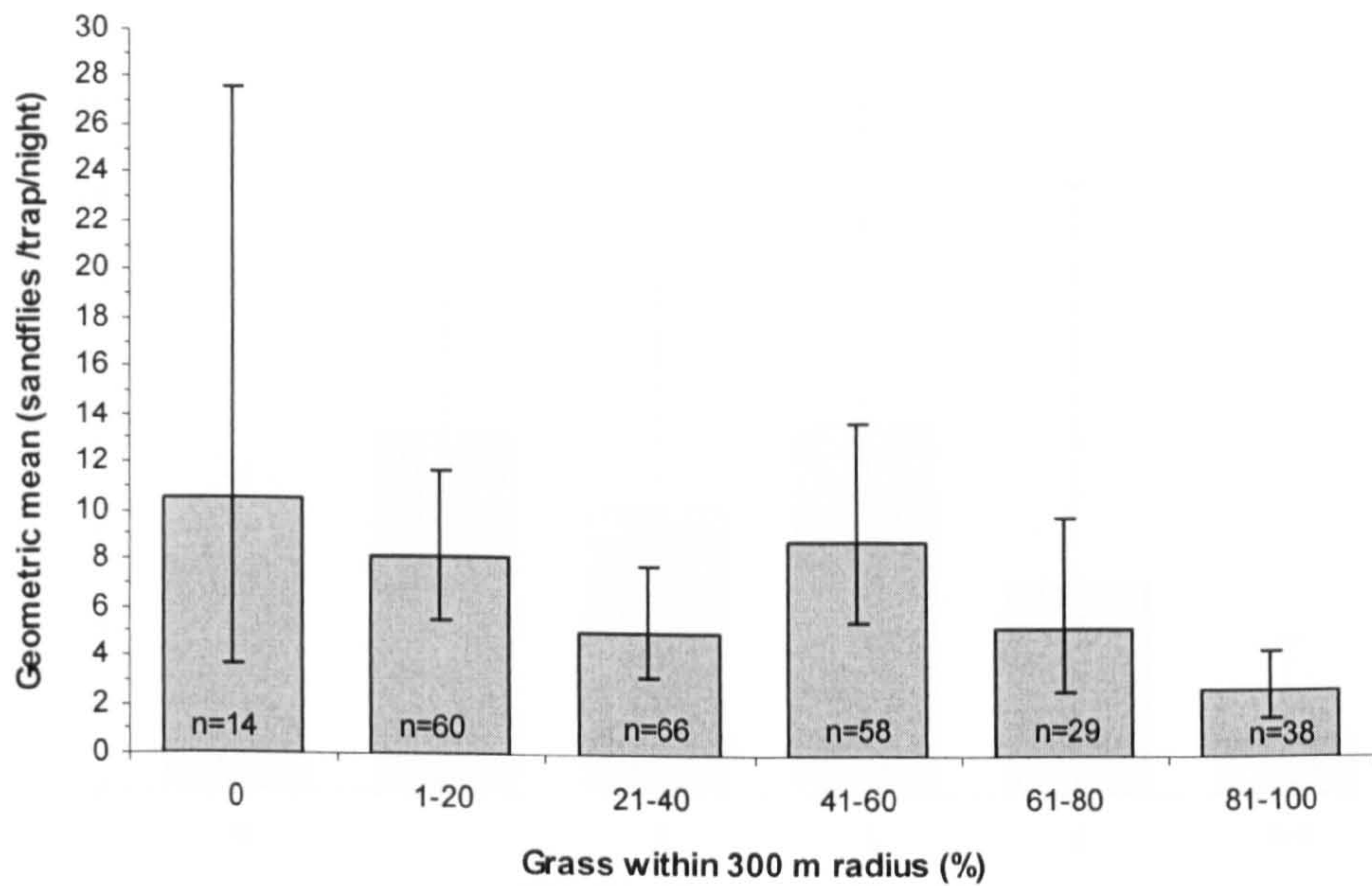


Figure 3.6 The relationship between percentage of land cover by grass within 300 m radius and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

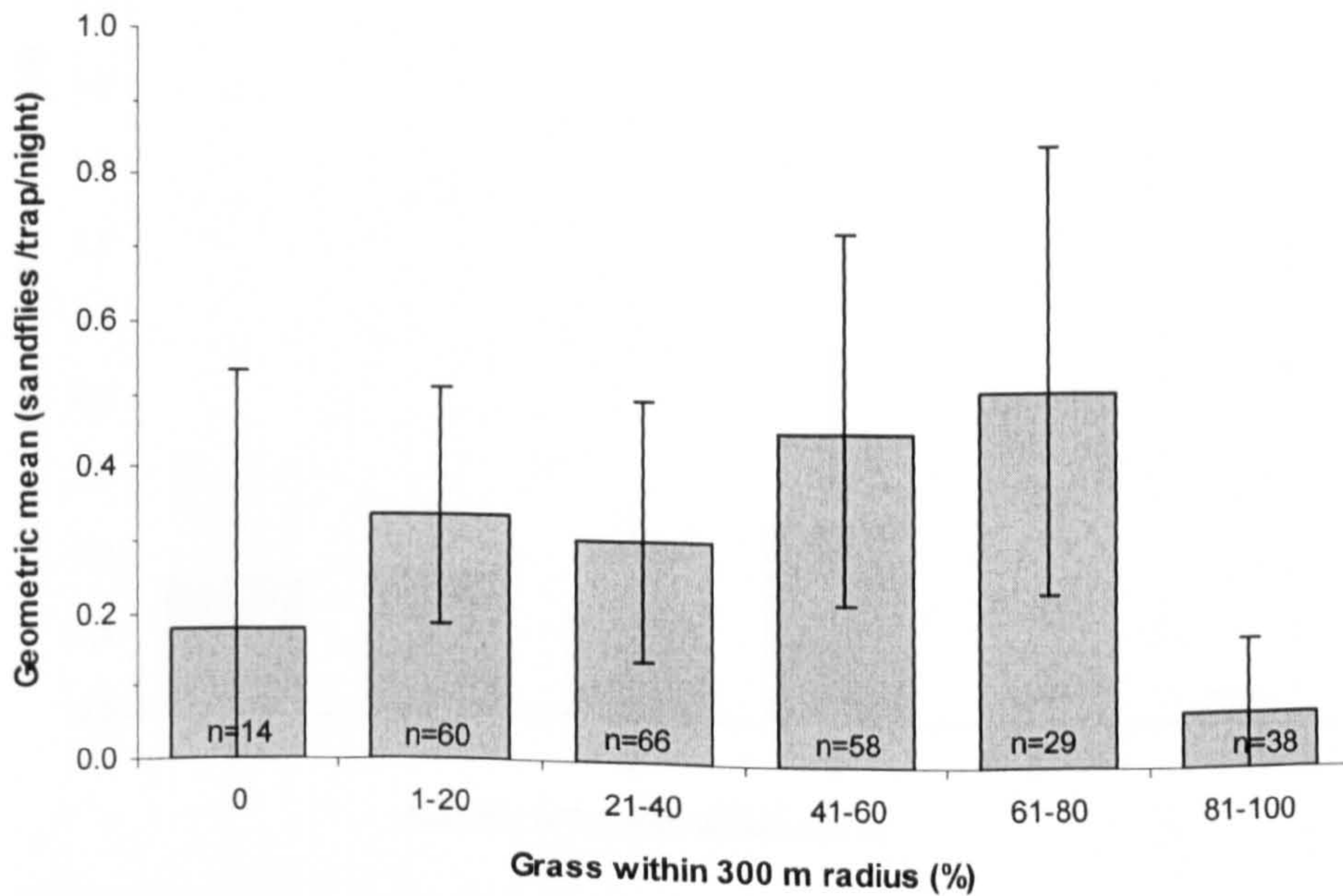


Figure 3.7 The relationship between percentage of land cover by grass within 300 m radius and *Lutzomyia nuneztovari* abundance inside houses (as measured by CDC light traps). Error bars as in Figure 3.6 .

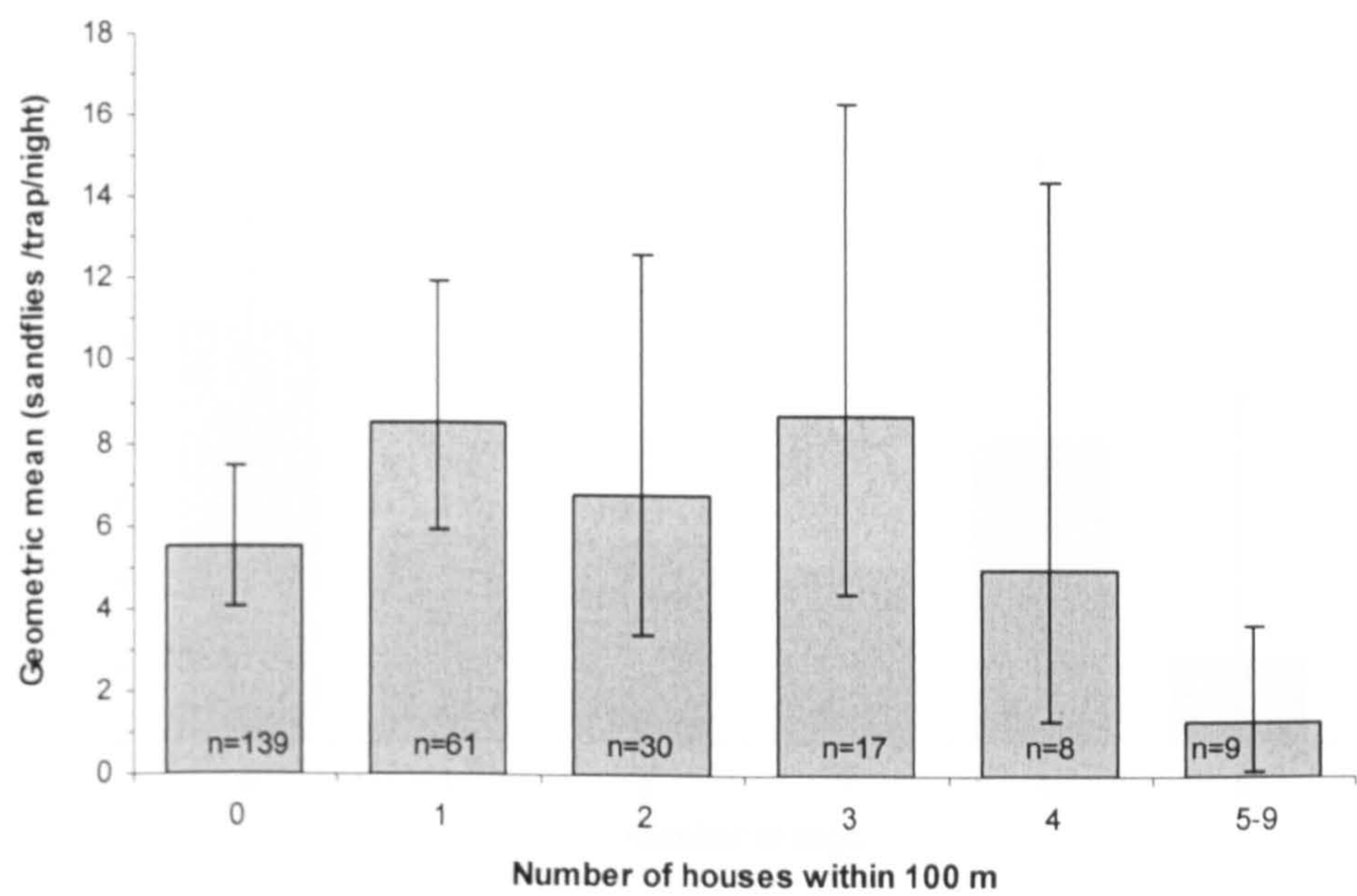


Figure 3.8 The relationship between the number of houses within 100 m radius and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

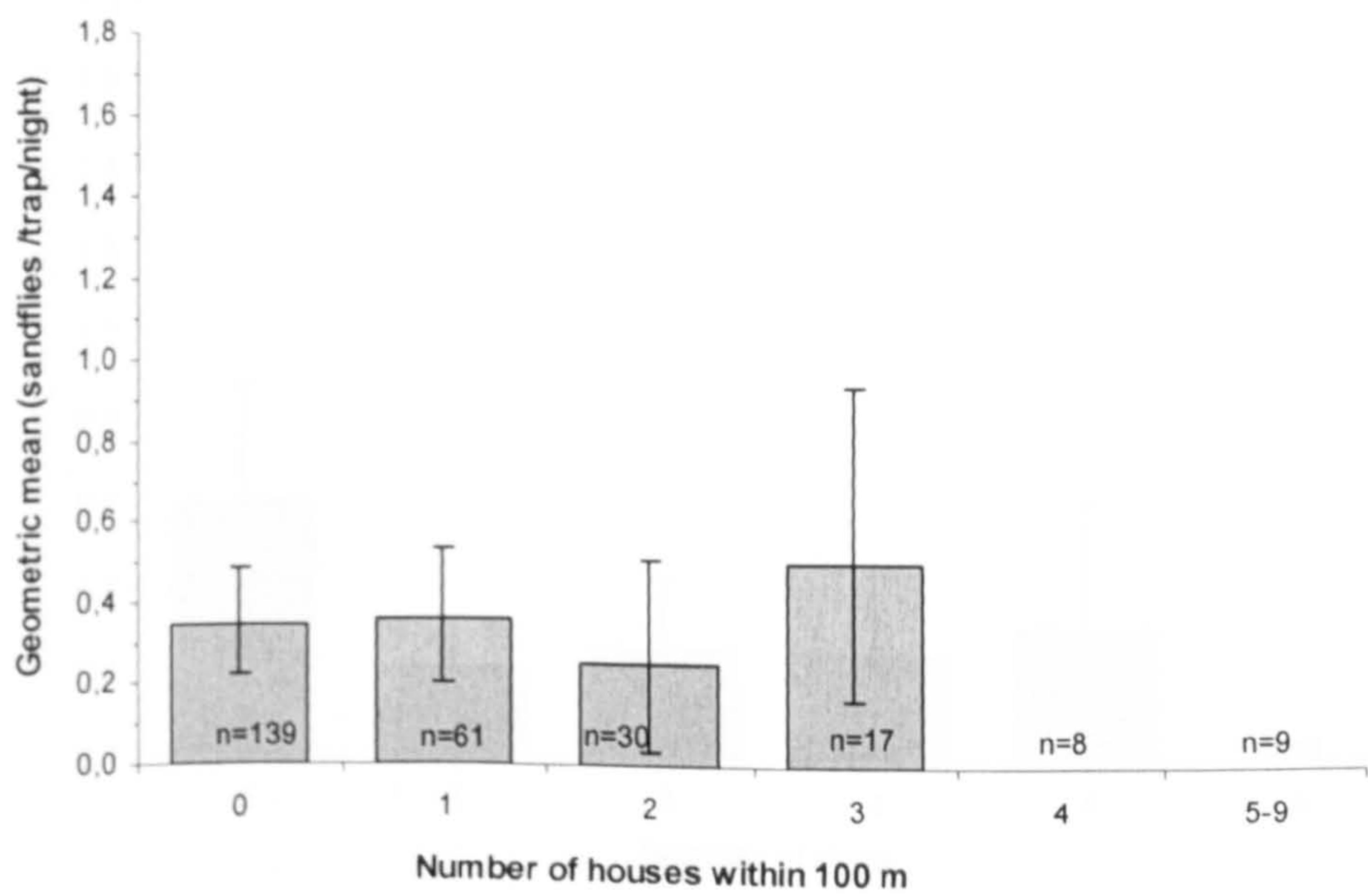


Figure 3.9 The relationship between number of houses within 100 m radius and *Lutzomyia nuneztovari* abundance (as measured by CDC light traps). Error bars as in Figure 3.8 .

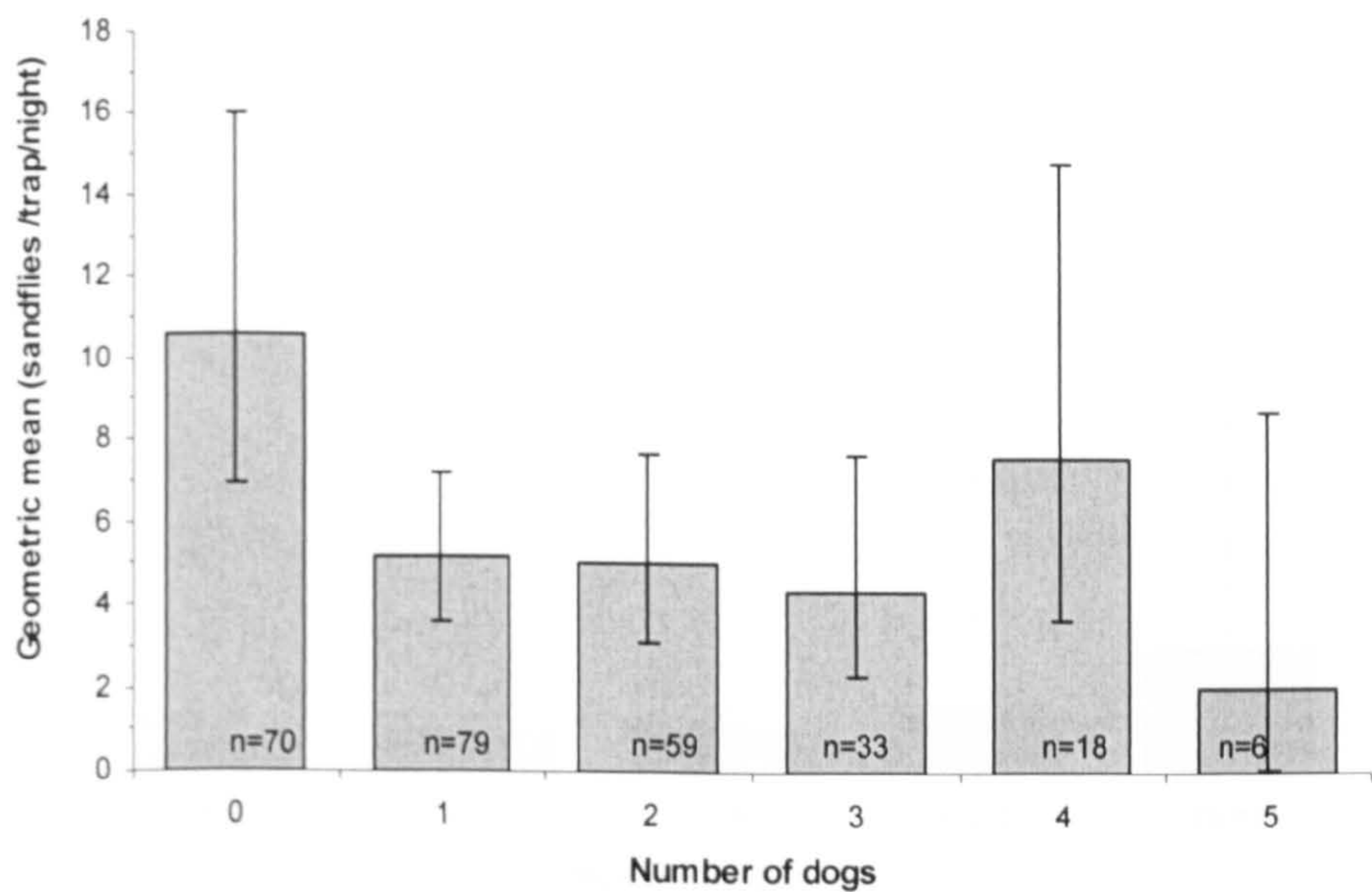


Figure 3.10 The relationship between the number of dogs per house and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

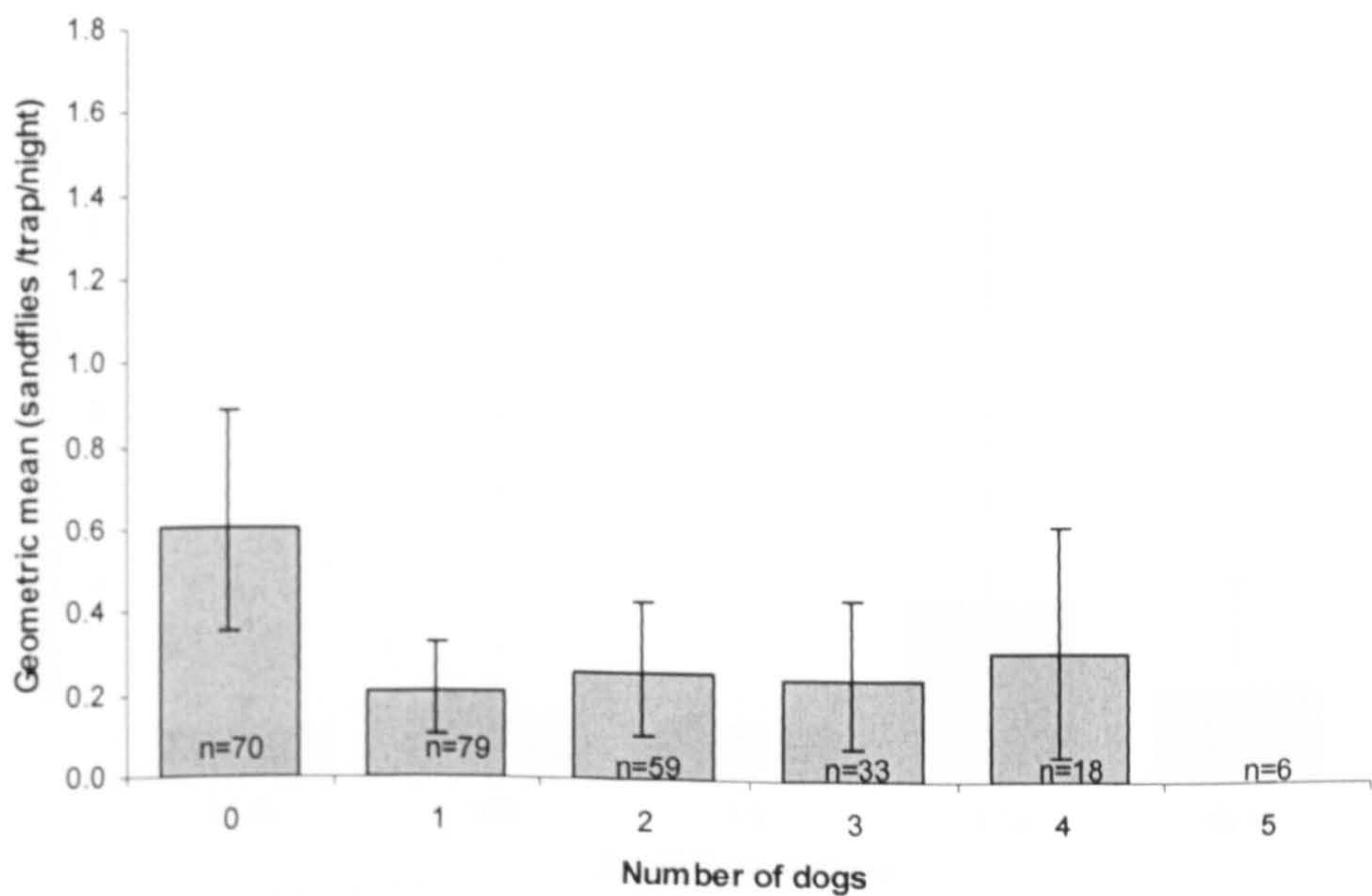


Figure 3.11 The relationship between number of dogs per house and *Lutzomyia nuneztovari* abundance (as measured by CDC light traps). Error bars as in Figure 3.10 .

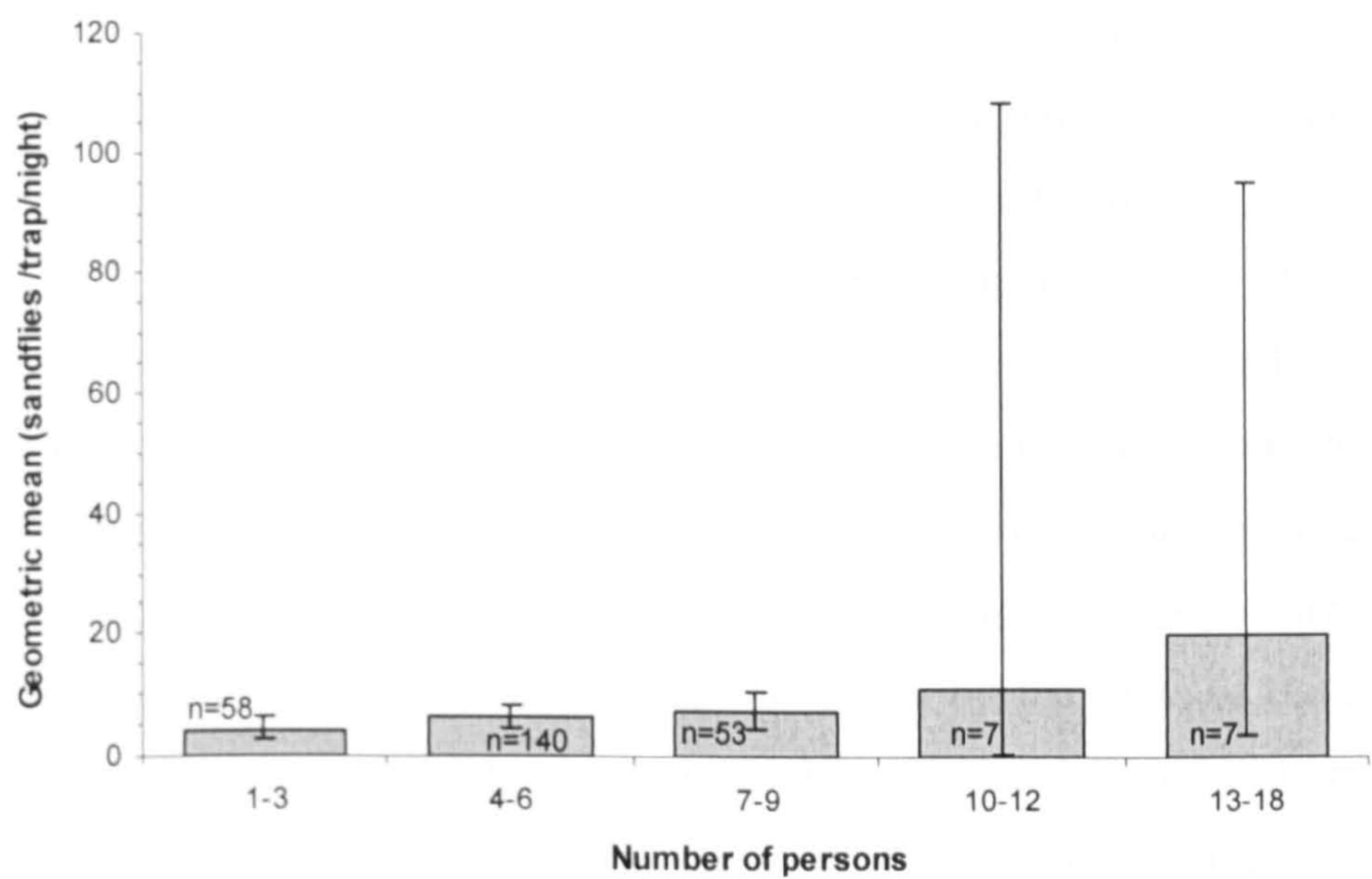


Figure 3.12 The relationship between the number of persons per house and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

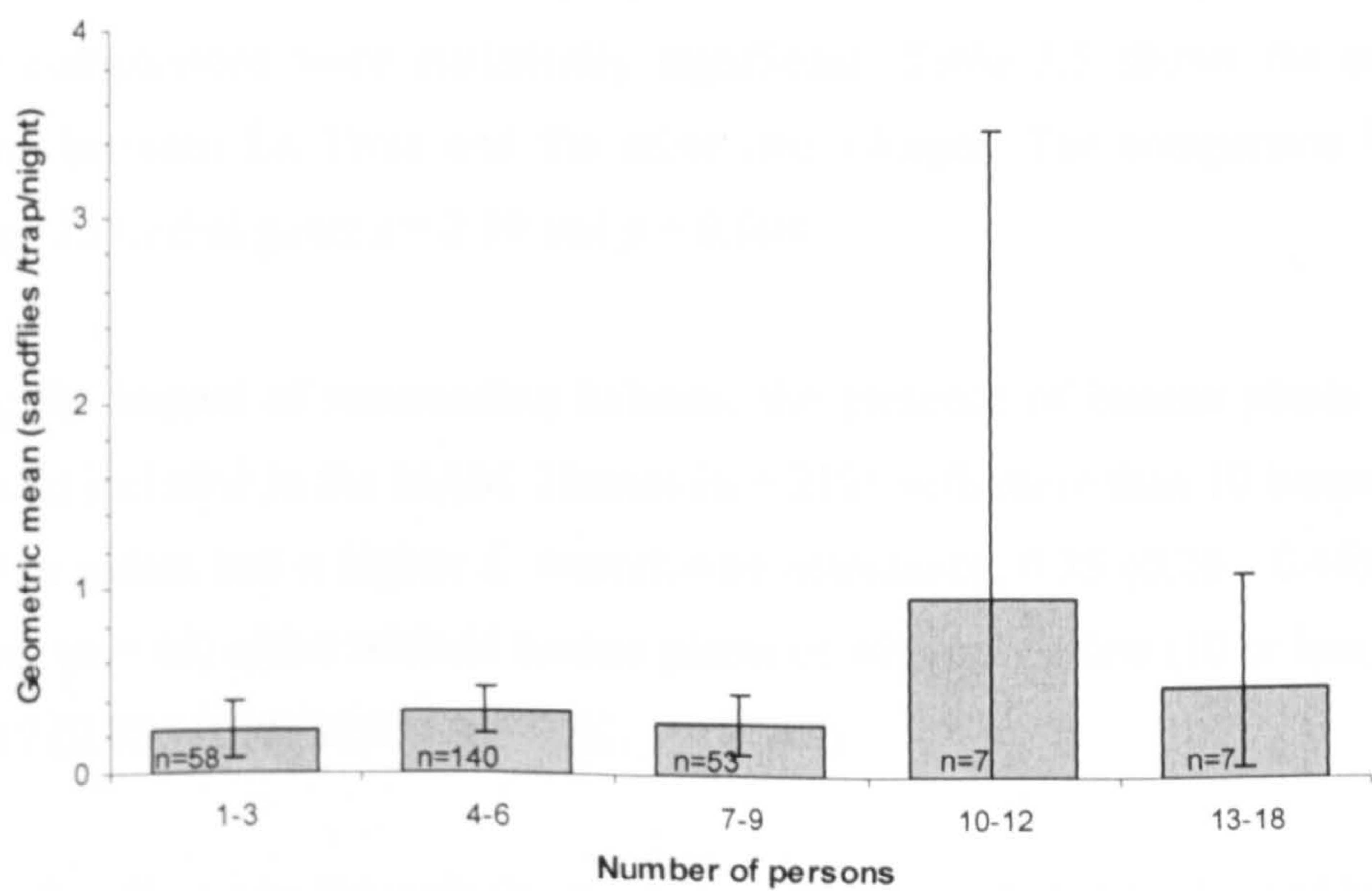


Figure 3.13 The relationship between number of persons per house and *Lutzomyia nuneztovari* abundance (as measured by CDC light traps). Error bars as in Figure 3.12 .

3.4.1.2 Risk factors for *Lutzomyia nuneztovari*

Stepwise elimination from a maximal model incorporating 34 risk factors for indoor *L. nuneztovari* raw abundance led to a MAM which included six variables: village; number of banana plants within 50 m; type of ceiling; number of dogs within 200 m; number of pigs within 200 m; and number of persons per house (Table 3.5). The MAM explained 16.9% of the household variance in indoor *L. nuneztovari* abundance (Annexe 37). The most important explanatory variable was the type of ceiling (which explained 7.9% of the sandfly variance), followed by village (5.2%), number of dogs (3.5%), number of pigs (2.3%), number of banana plants (1.3%) and number of persons/house (0.9%). The goodness of fit of the model was tested by the plots of the Anscombe residuals, which are recommended for negative binomial models and that are expected to closely follow a normal distribution, against the fitted values (Annexe 38) and the plot of the predicted against the observed abundance (Annexe 39).

Comparing villages, the abundance of *L. nuneztovari* followed a similar pattern to that recorded for *L. longiflocosa*, with highest abundance in La Troja, 0.58 s/LT/n, followed by El Cedral, 0.28 s/LT/n, and finally by Brasilia with 0.15 s/LT/n (Annexe 30). All pair wise comparisons were statistically significant. Table 3.5 shows the statistical comparison between La Troja and the other two villages. The comparison between Brasilia and El Cedral gave: $z = 2.90$ and $p = 0.004$.

Regarding the impact of surrounding habitats, the presence of banana plants was the only variable included in the MAM. Houses ($n = 219$) with more than 10 banana plants within 50 m radius had a higher *L. nuneztovari* abundance, 0.35 (0.26 - 0.46) s/LT/n, than houses ($n = 46$) either without banana plants or with only a few (10 or less) banana plants, 0.17 (0.05 - 0.30) s/LT/n ($z = 2.61$, $p = 0.009$).

The type of ceiling was the only house feature finally included in the MAM model. Houses with ceilings classified as "close plank" had the highest indoor abundance of *L. nuneztovari*, 0.51 (0.28 - 0.78) s/LT/n. This value was twice the abundance recorded for any of the other three types of ceiling: close plank and hole, 0.22 (0.07 - 0.39) s/LT/n, plank with spaces, 0.24 (0.10 - 0.39) s/LT/n, and no ceiling, 0.26 (0.14 - 0.38) s/LT/n. Nevertheless, significant differences were found only when "close plank" was compared with "no ceiling" ($z = -3.13$, $p = 0.002$) (Figure 3.15).

Table 3.5 Risk factors for indoor *Lutzomyia nuneztovari* abundance (raw number/CDC trap/house-night) identified by multivariate analysis.

Explanatory variable		Coefficient	S.E.	z	P>z	95% C.I.	
						Min	Max
Village	La Troja ^a						
	Brasilia	-1.986	0.378	-5.25	<0.001	-2.727	-1.245
	El Cedral	-0.838	0.325	-2.58	0.010	-1.474	-0.201
Surrounding habitats features							
Number of banana plants (within 50 m)							
0 - 10 ^a							
> 10		1.061	0.407	2.61	0.009	0.263	1.859
House features							
Type of ceiling							
Close plank ^a							
Close plank and hole		-0.494	0.496	-1	0.319	-1.466	0.477
Plank with spaces		-0.436	0.383	-1.14	0.255	-1.187	0.314
No ceiling		-1.105	0.353	-3.13	0.002	-1.796	-0.414
Potential hosts							
Number of dogs (within 200 m)		-0.501	0.116	-4.3	<0.001	-0.729	-0.273
Number of pigs (within 200 m)		-0.480	0.194	-2.47	0.013	-0.860	-0.099
Number of persons per house		0.106	0.050	2.13	0.033	0.008	0.204
Intercept		-0.068	0.503	-0.14	0.892	-1.054	0.917

^aBaseline Category.

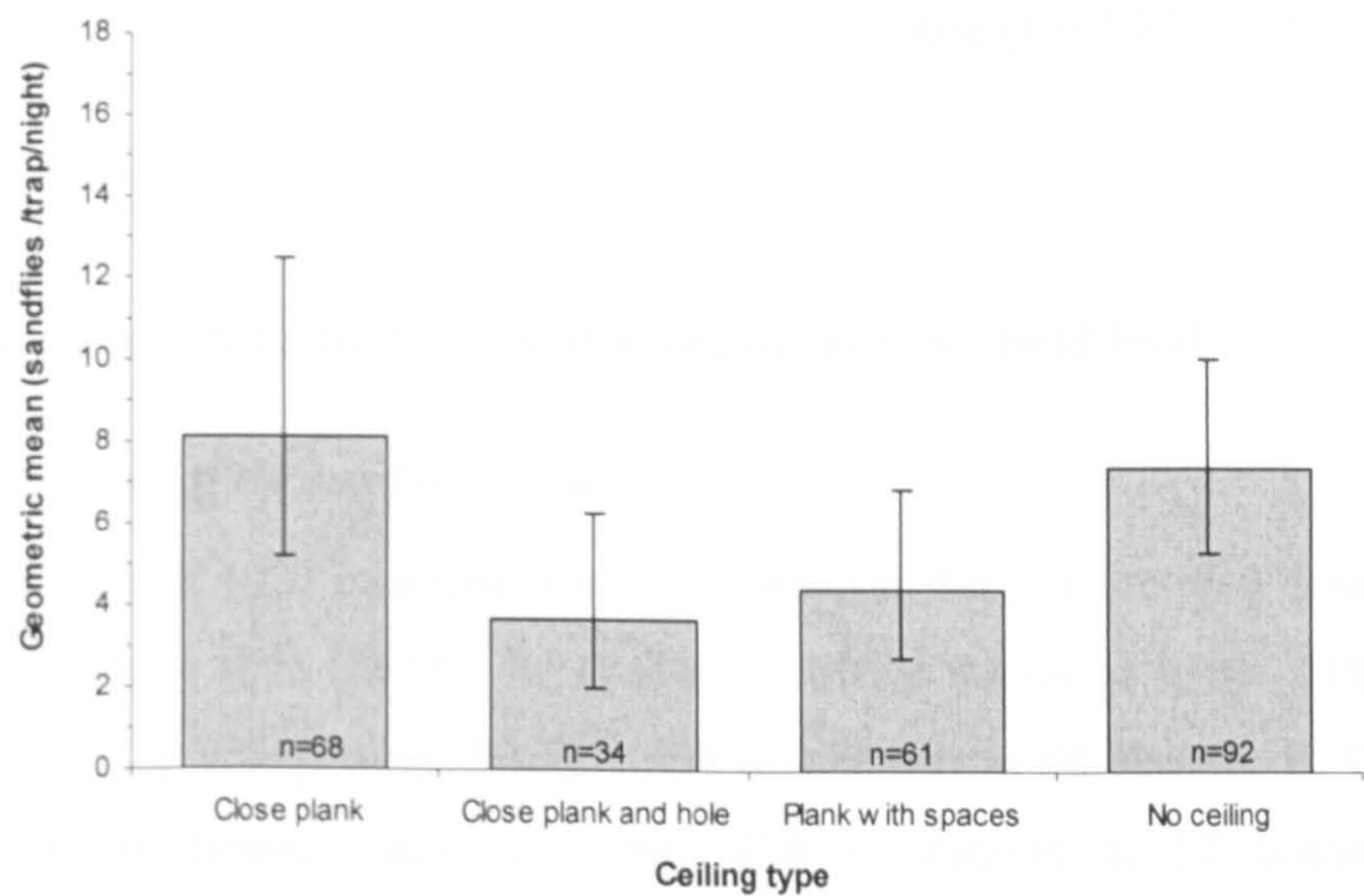


Figure 3.14 The relationship between type of ceiling and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

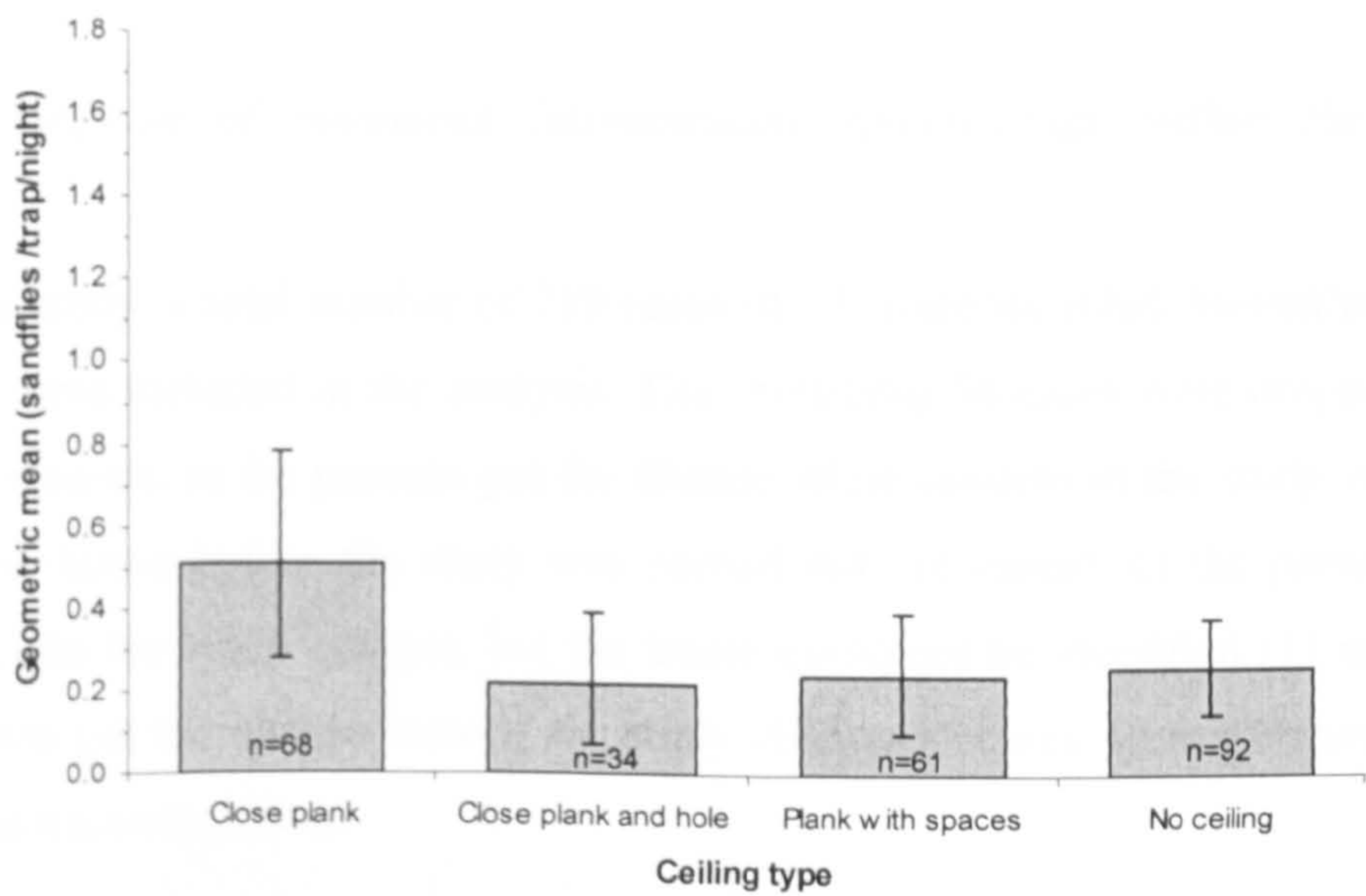


Figure 3.15 The relationship between ceiling type and *Lutzomyia nuneztovari* abundance inside houses (as measured by CDC light traps). Error bars as in Figure 3.14 .

Regarding the impact of potential blodmeal sources, the number of both dogs and pigs within 200 m radius from the house had a negative relationship with indoor abundance of *L. nuneztovari* ($z = -4.30, p < 0.001$; $z = -2.47, p = 0.013$, respectively) (Figures 3.11 and 3.17, respectively). In contrast, the indoor abundance of *L. nuneztovari* was

positively associated with the number of persons per house ($z = 2.13$, $p = 0.033$) (Figure 3.13).

3.4.2 Risk factors for cutaneous leishmaniasis at household level

3.4.2.1 Description of the sampled population

A total population of 1427 inhabitants were recorded in the 271 sampled houses, located at a mean altitude of 1655 (1637 - 1672) m a.s.l., within the range 1310 - 2180 m a.s.l. . The distribution by village was: Brasilia 554 inhabitants in 98 houses; El Cedral, 445 inhabitants in 86 houses, and La Troja, 428 inhabitants in 87 houses. Gender composition was male biased with 54.7% (781 / 1,427) males, with the same bias in each area. The population was formed mainly by young people with 66.4% (943 / 1,420) aged 30 years or less, and 25.8% (367 / 1,420) with age 9 years or less (seven persons had missing data for age).

3.4.2.2 Description of cutaneous leishmaniasis epidemiology within the sampled villages

During the study, a total number of 219 cases of CL were recorded. Nevertheless, only 163 cases were included in the analysis. The remaining 56 cases were dropped for the following reasons: a) the persons got the disease while resident in the study village, but had left the house before the study was carried out (38 cases), b) the person got the disease within the study villages, but the house could not be identified (11 cases); and c) the person got the disease outside the study villages (7 cases, from villages of Neiva and Baraya municipalities).

Table 3.6 classifies the 163 CL cases on clinical and diagnostic grounds. Most of the cases were cured cases, 85.9% (140 / 163), and almost all had reportedly occurred within the last 8 years, 96.9% (158 / 163). Confirmation that the disease was CL, according to the criteria described previously, was achieved in 91.4 % (149 / 163) of cases. The remaining unconfirmed cases, 8.6% (14 / 163), comprised "cured unconfirmed" (6 cases) or "new unconfirmed" cases (8 cases). Validation of confirmed

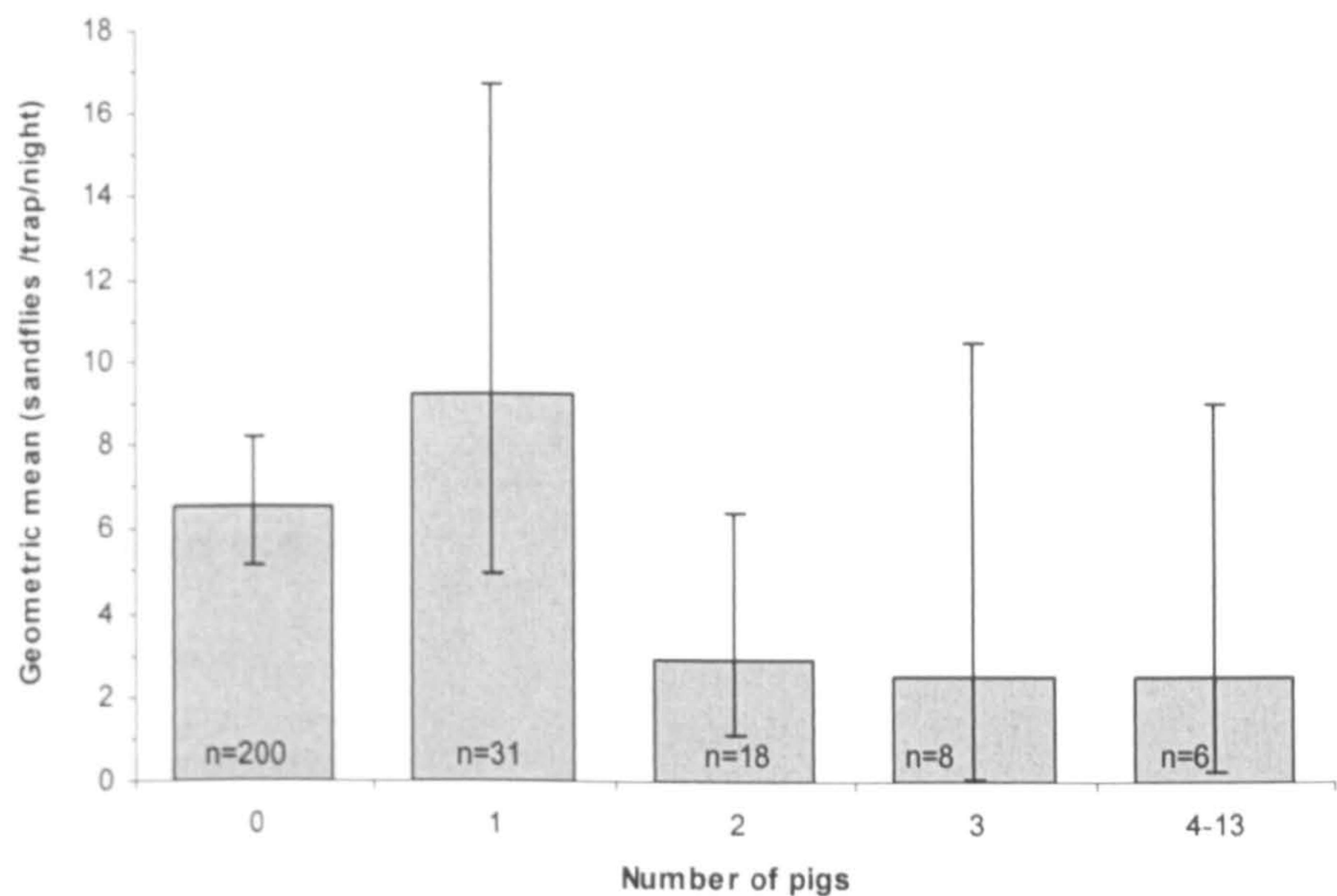


Figure 3.16 The relationship between number of pigs and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps). Error bars are the 95% confidence intervals around the geometric means.

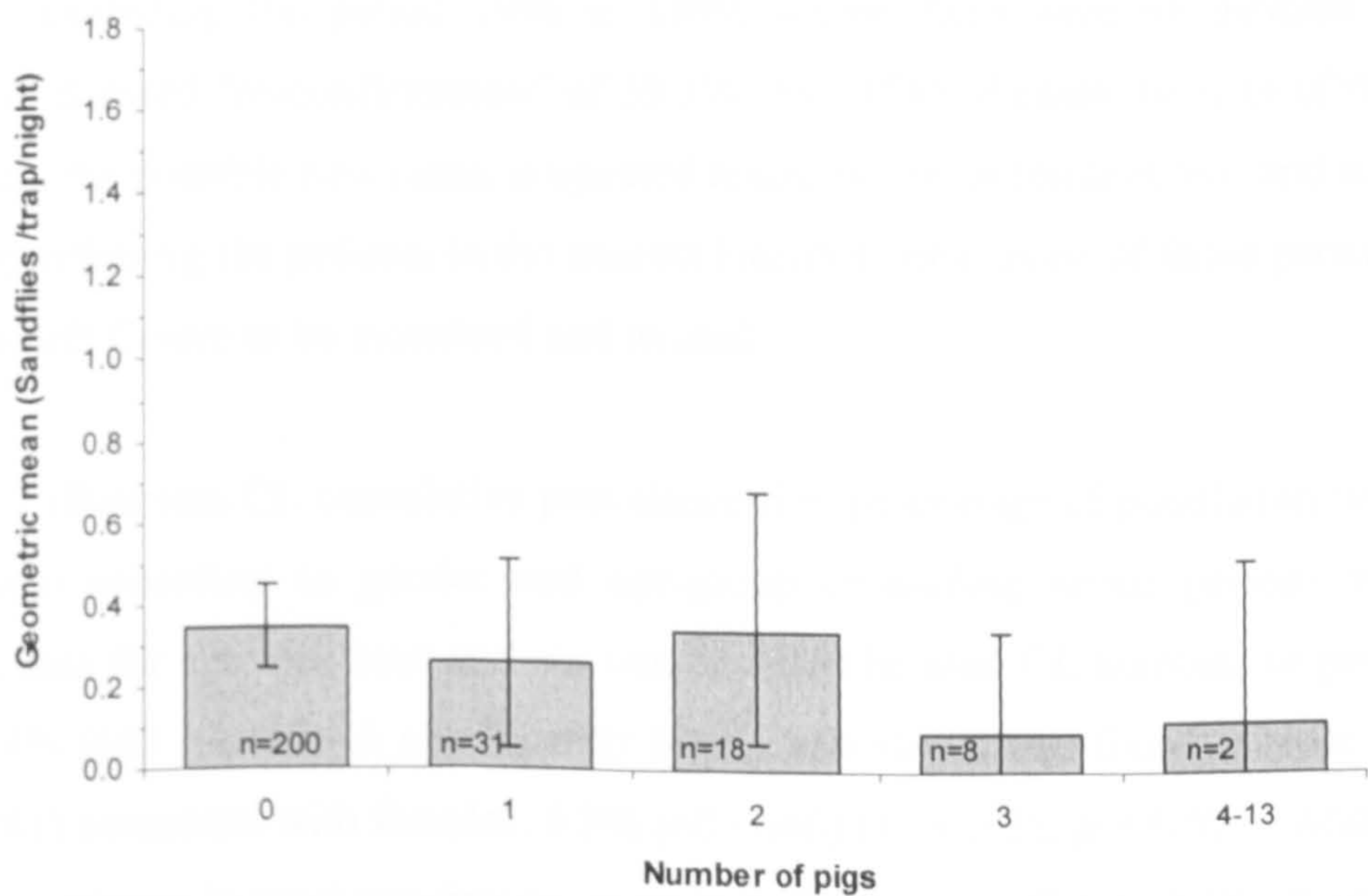


Figure 3.17 The relationship between number of pigs and *Lutzomyia nuneztovari* abundance inside houses (as measured by CDC light traps). Error bars as in Figure 3.16 .

Table 3.6 Summary of all types of cutaneous leishmaniasis cases detected during the risk factors study in the three sampled villages of Huila department.

Type of case	Village							
	La Troja		Brasilia		El Cedral		Total	
	No.	%	No.	%	No.	%	No.	%
Cured (confirmed CL)	48	90.6	69	85.2	23	79.3	140	85.9
Cured unconfirmed	3	5.7	3	3.7	0		6	3.7
New unconfirmed	1	1.9	3	3.7	4	13.8	8	4.9
Suspected mucocutaneous (confirmed CL)	0		2	2.5	0		2	1.2
Reactivation or reinfection (confirmed CL)	1	1.9	0		0		1	0.6
Suspected reactivation or reinfection (confirmed CL)	0		4	4.9	2	6.9	6	3.7
Total	53	100	81	100	29	100	163	100

cases against the epidemiological reports held by the Huila Health Service, SSDH (1979 to 2001, excluding the period 1996 to 1999, where there were no detailed records available) allowed “re-confirmation” of 59.3% (64 / 108) of cases. In spite of the effort to confirm the possible new cases, suspected reactivations or reinfections, and suspected MCL, by referring the patients to the nearest Health Centre, none of those patients went to the Health Centre to be examined and treated.

Table 3.7 illustrates CL cumulative prevalence (i.e. percentage of population with scars or lesions) according to gender and age-group (excluding seven persons who had missing data for age: one case and six non-cases). The total CL cumulative prevalence was 11.4% (163 / 1,427). A significantly higher prevalence was found in males, 13.2% (103 / 781) compared with females, 9.3% (60 / 646) ($X^2 = 5.32, p = 0.021$). Males had a higher prevalence in most age-groups, even in the early ages (Figure 3.18). With respect to CL cumulative prevalence by age, the pattern suggests a sharp increase in early ages reaching a plateau by the age of about 8 years, and prevalence apparently dropping to zero amongst elderly inhabitants (> 70 years old) (Annexe 40). Dividing the data into 11 age-groups (Table 3.7) it is clearer that CL cumulative prevalence was evenly distributed by age (between 9.2 - 12.6%), except for age groups 3.1 - 6, 9.1 - 11 and

Table 3.7 Cutaneous leishmaniasis cumulative prevalence (confirmed plus unconfirmed cases) by gender and age-group detected in the three sampled villages.

Age-group	Sex	Village							
		La Troja (n=87)		Brasilia (n=98)		El Cedral (n=86)		Total (n=271)	
		Ca./Po. ^a	%	Ca./Po.	%	Ca./Po.	%	Ca./Po.	%
0-3	Female	1/20	5.0	2/24	8.3	0/16	0	3/60	5.0
	Males	2/16	12.5	6/22	27.3	0/22	0	8/60	13.3
	Total	3/36	8.3	8/46	17.4	0/38	0	11/120	9.2
3.1-6	Female	0/13	0	3/29	10.3	3/17	17.6	6/59	10.2
	Males	1/14	7.1	9/27	33.3	1/16	6.3	11/57	19.3
	Total	1/27	3.7	12/56	21.4	4/33	12.1	17/116	14.7
6.1-9	Female	3/20	15.0	3/18	16.7	2/22	9.1	8/60	13.3
	Males	1/19	5.3	4/30	13.3	0/22	0	5/71	7.0
	Total	4/39	10.3	7/48	14.6	2/44	4.5	13/131	9.9
9.1-11	Female	2/9	22.2	3/16	18.8	0/7	0	5/32	15.6
	Males	3/6	50.0	2/14	14.3	0/11	0	5/31	16.1
	Total	5/15	33.3	5/30	16.7	0/18	0	10/63	15.9
11.1-15	Female	4/20	20.0	1/26	3.8	2/13	15.4	7/59	11.9
	Males	6/22	27.3	6/25	24.0	1/15	6.7	13/62	21.0
	Total	10/42	23.8	7/51	13.7	3/28	10.7	20/121	16.5
15.1-20	Female	2/19	10.5	4/35	11.4	1/30	3.3	7/84	8.3
	Males	5/25	20.0	6/34	17.6	2/16	12.5	13/75	17.3
	Total	7/44	15.9	10/69	14.5	3/46	6.5	20/159	12.6
20.1-25	Female	1/12	8.3	2/22	9.1	1/19	5.3	4/53	7.5
	Males	2/28	7.1	3/27	11.1	3/22	13.6	8/77	10.4
	Total	3/40	7.5	5/49	10.2	4/41	9.8	12/130	9.2
25.1-30	Female	1/11	9.1	3/17	17.6	0/13	0	4/41	9.8
	Males	1/15	6.7	7/25	28.0	0/22	0	8/62	12.9
	Total	2/26	7.7	10/42	23.8	0/35	0	12/103	11.7
30.1-40	Female	1/17	5.9	5/27	18.5	2/19	10.5	8/63	12.7
	Males	2/26	7.7	7/35	20.0	0/34	0	9/95	9.5
	Total	3/43	7.0	12/62	19.4	2/53	3.8	17/158	10.8
40.1-50	Female	3/22	13.6	0/16	0	0/21	0	3/59	5.1
	Males	3/15	20.0	3/27	11.1	3/22	13.6	9/64	14.1
	Total	6/37	16.2	3/43	7.0	3/43	7.0	12/123	9.8
>50.1	Female	2/29	6.9	1/18	5.6	2/27	7.4	5/74	6.8
	Males	6/49	12.2	1/37	2.7	6/36	16.7	13/122	10.7
	Total	8/78	10.3	2/55	3.6	8/63	12.7	18/196	9.2
Total	Female	20/192	10.4	27/248	10.9	13/204	6.4	60/644 ^b	9.3
	Males	32/235	13.6	54/303	17.8	16/238	6.7	102 ^d /776 ^c	13.1
	Total	52/427	12.2	81/551	14.7	29/442	6.6	162 ^e /1420 ^f	11.4

^a Ca./Po. = Cases / population; ^{b, c, d} Persons without age data which were not included in the table: Two, five and one, respectively; ^{e, f} Total number of person not included: One and seven, respectively.

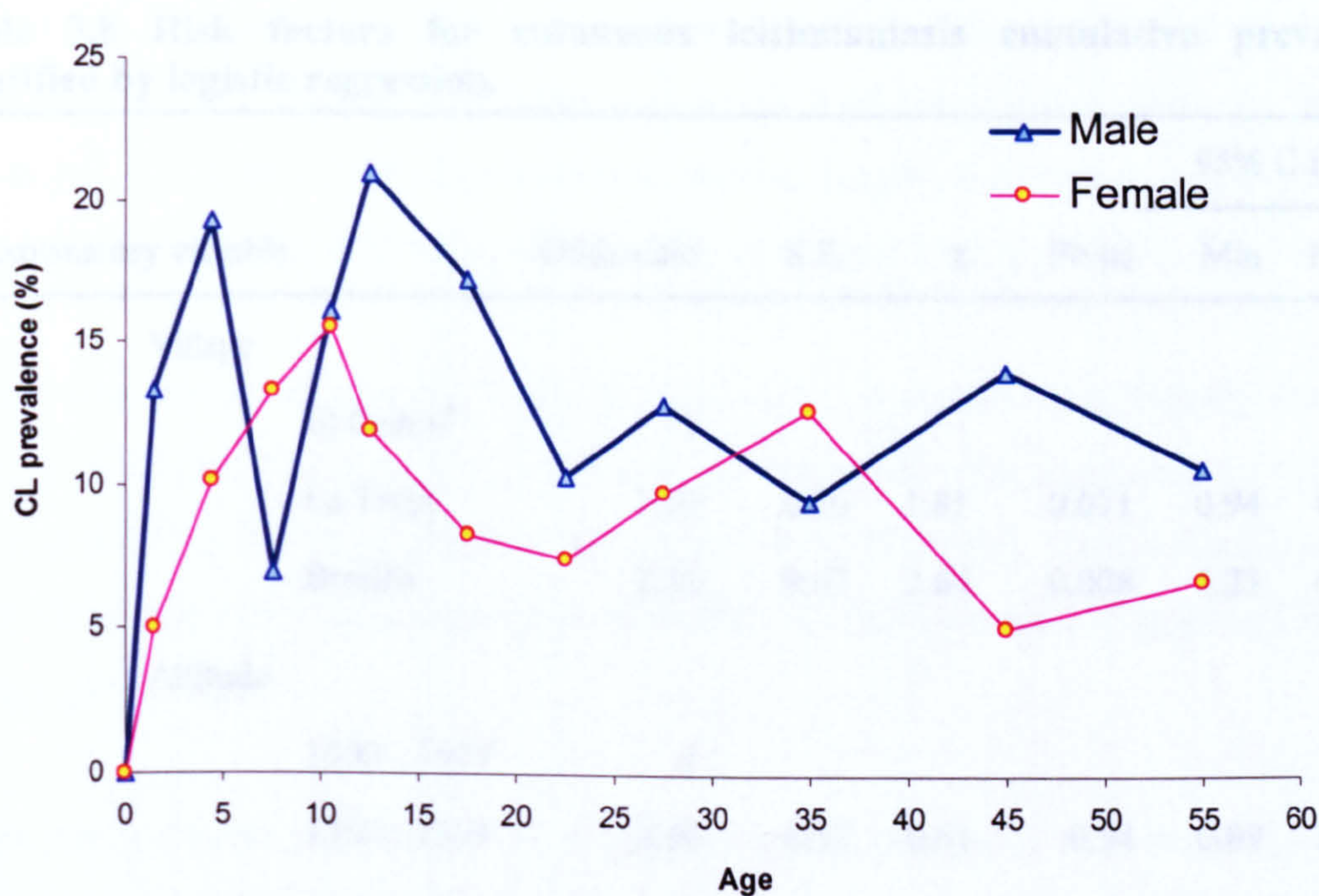


Figure 3.18 Cumulative prevalence of cutaneous leishmaniasis by gender and age groups (see Table 3.7).

11.1 - 15 years where the prevalence seemed higher, 14.7%, 15.9% and 16.5%. Comparison of 5 age-groups (0 - 5.9, 6 - 14.9, 15 - 29.9, 30 - 49.9, and > 50) showed no significant differences between groups ($X^2_{(4)} = 2.99, p = 0.572$).

Comparing villages, Brasilia had the highest CL cumulative prevalence, 14.6% (81 / 554), followed by La Troja, 12.4% (53 / 428), and El Cedral, 6.6% (29 / 445). The prevalence in El Cedral was significantly lower than the prevalence in Brasilia, ($X^2 = 15.72, p < 0.001$), or La Troja ($X^2 = 8.15, p = 0.004$). No differences were found in prevalence between Brasilia and La Troja ($X^2 = 0.84, p = 0.358$).

3.4.2.3 Risk factors for cutaneous leishmaniasis cumulative prevalence

The multivariate analysis for risk factors of CL cumulative prevalence, clustered by household, detected the following significant variables: village; altitude; gender; length of residence in the house; and house type (Table 3.8). The MAM explained 10.3% of the variance in CL risk (Annexe 41). The most important variables, taking into account their explanatory power were, in decreasing order: altitude (which explained 5.0% of

Table 3.8 Risk factors for cutaneous leishmaniasis cumulative prevalence identified by logistic regression.

Explanatory variable	Odds ratio	S.E.	z	P> z	95% C.I.		
					Min	Max	
Village							
El Cedral ^a	1						
La Troja	1.99	0.76	1.81	0.071	0.94	4.21	
Brasilia	2.22	0.67	2.64	0.008	1.23	4.02	
Altitude							
1500 - 1599 ^a	1						
1300 - 1399	0.56	0.53	-0.61	0.54	0.09	3.58	
1400 - 1499	0.43	0.17	-2.15	0.032	0.20	0.93	
1600 - 1699	0.50	0.18	-1.95	0.051	0.24	1.00	
1700 - 1799	0.23	0.09	-3.74	<0.001	0.11	0.50	
1800 - 1899	0.21	0.11	-3.05	0.002	0.07	0.57	
1900 - 2200	0.08	0.08	-2.43	0.015	0.01	0.61	
Demographic features							
Length of residence (per year)	1.03	0.01	4.54	<0.001	1.02	1.04	
Gender							
Female ^a	1						
Male	1.46	0.25	2.2	0.028	1.04	2.05	
House features							
House type							
House ^a	1						
Hut	0.37	0.183	-2.01	0.044	0.14	0.98	

^aBaseline category.

the variance); length of residence in the house (2.0%); village (1.2%); house type (0.8%); and gender (0.5%).

The MAM confirmed a positive association between male gender and cumulative prevalence of CL. The risk of CL for males was 1.46 times greater than for females ($p = 0.028$, C.I. 1.04 - 2.05). In addition to gender, length of residence in the house, as expected, increases the odds of cumulative CL by 3% per year ($p < 0.001$, C.I. 1 - 4%).

Village was confirmed as a risk factor for CL. Living in Brasilia significantly increased the risk of CL by 2.22 (C.I. 1.22 - 4.02) times compared with El Cedral, ($p = 0.008$). The risk of CL in La Troja village was greater than in El Cedral, but not significantly so ($p = 0.071$); nor was there a significant difference in risk between La Troja and Brasilia village ($p = 0.745$).

There was a decrease in risk for all altitudinal ranges compared with the range 1500 - 1600 m a.s.l., where the highest prevalence of CL was recorded (Figure 3.19), with significant differences compared to most of the other ranges: 1400 - 1500 m a.s.l., odds ratio 0.43; 1700 - 1800 m a.s.l., odds ratio 0.23; 1800 - 1900 m a.s.l. , odds ratio 0.21; and 1900 - 2200 m a.s.l., odds ratio 0.08. Comparison of the range 1500 - 1600 m a.s.l. with the range 1600 - 1700 m a.s.l. , odds ratio 0.50, had border-line significance ($p = 0.05$); no significant difference was found in comparison with the range 1300 - 1400 m a.s.l. .

People living in huts had surprisingly much lower CL prevalence, 3.6% (7 / 196, C.I. was not calculated as "np < 10" where n= sample size, and p= proportion of cases) than people living in houses, 12.7% (156 / 1,231, C.I.: 10.8 – 14.5), but the difference had only borderline significance ($p = 0.04$) (Table 3.8).

3.4.3 Correlation between the risk of CL transmission and the abundance, inside houses, of *Lutzomyia longiflocosa* and *Lutzomyia nuneztovari*

Univariate logistic analysis of cutaneous leishmaniasis prevalence, clustering by household, showed a very highly significant positive relationship with the abundance of *L. longiflocosa* females (applying the LRT, $X^2 = 15.84$, $p = 0.0001$). The same analysis found no significant relationship between CL prevalence and the abundance of *L. nuneztovari* females ($X^2 = 0.08$, $p = 0.778$). Figures 3.20 and 3.21 illustrate these results showing the histograms of sandfly female abundance (categorized by ranges of

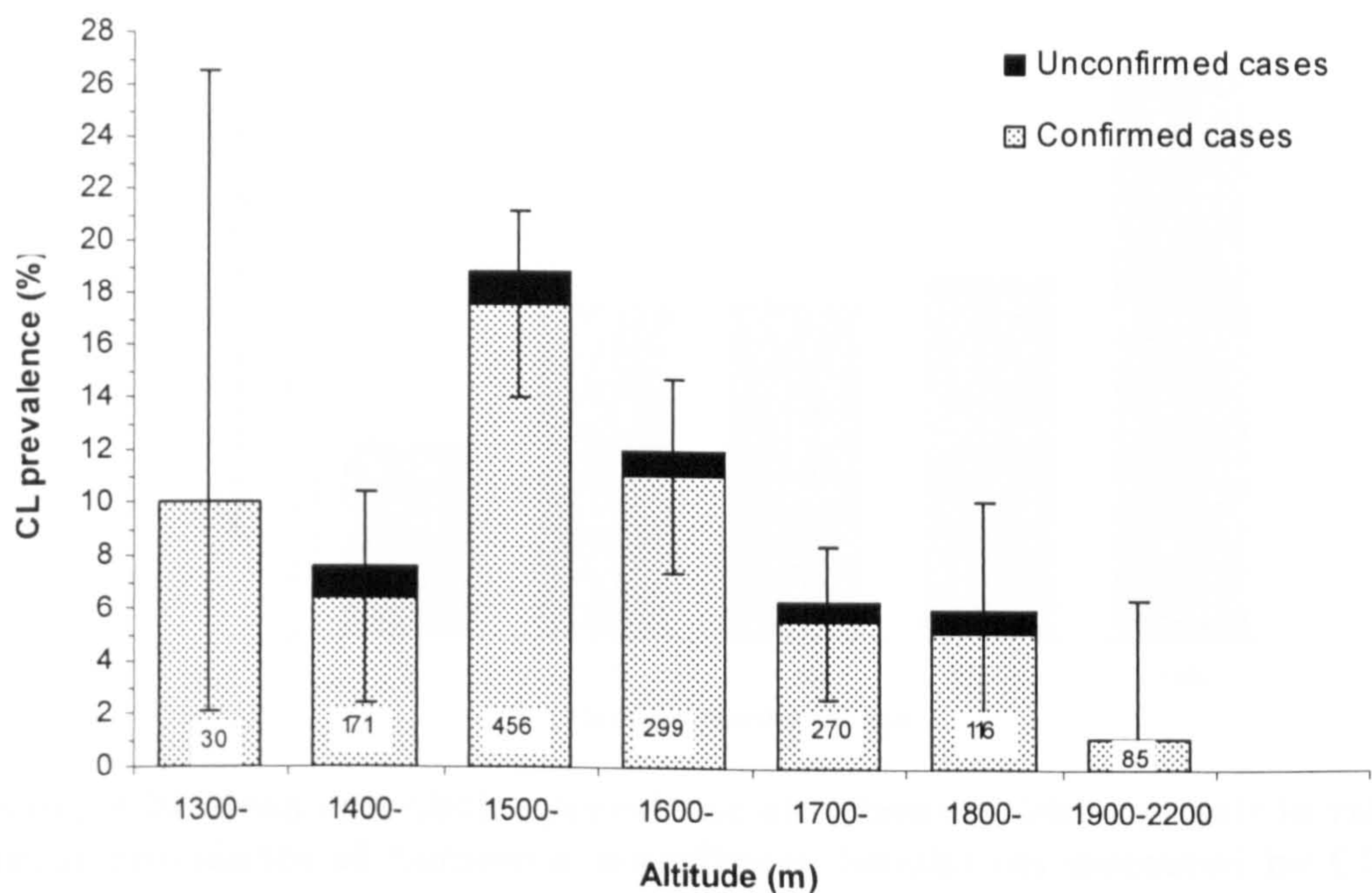


Figure 3.19 Cutaneous leishmaniasis cumulative prevalence by altitude (confirmed cases = 149; total cases = 163; total population = 1427). Error bars are the 95% confidence intervals. Numbers on the bottom of each bar show population size for the respective altitudinal range.

abundance) against the mean cumulative prevalence of CL per house for *L. longiflocosa* and *L. nuneztovari*, respectively.

The same pattern was observed when total time of residence in the house and time of residence per year (> 3 months) were included in the logistic model to control for length of exposure to the disease. *L. longiflocosa* abundance still presented a highly significant positive relationship with CL prevalence ($X^2 = 11.80, p = 0.006$), while *L. nuneztovari* abundance was unrelated to disease risk ($X^2 = 0.5, p = 0.479$). When the two species were included in the logistic model both were significant, but in opposing directions (Table 3.9). *L. longiflocosa* kept its highly significant positive relationship with CL prevalence, with an increase of 1.38 times (1.11 - 1.73), or 38%, in CL prevalence for each unitary increase in the log number of *L. longiflocosa* females ($X^2 = 22.64, p < 0.001$), i.e. for each 2.7 times increase in *L. longiflocosa* abundance. In contrast, *L. nuneztovari* was negatively associated with CL prevalence, which decreased by 50% for each 2.7 times increase in *L. nuneztovari* abundance ($X^2 = 11.34, p = 0.001$).

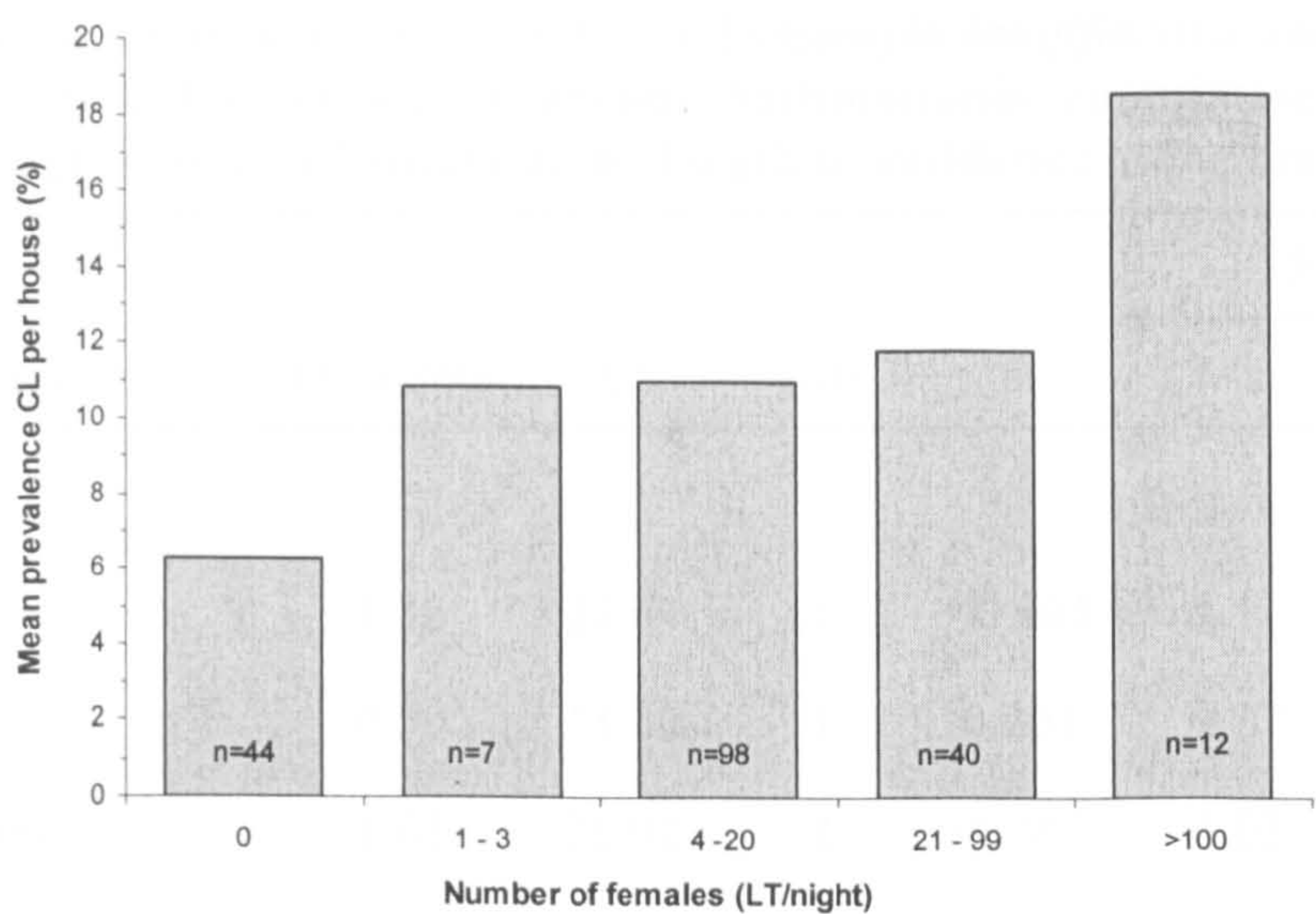


Figure 3.20 Mean cumulative prevalence of cutaneous leishmaniasis in relation to indoor abundance of *Lutzomyia longiflocosa* females (as measured by CDC light traps).

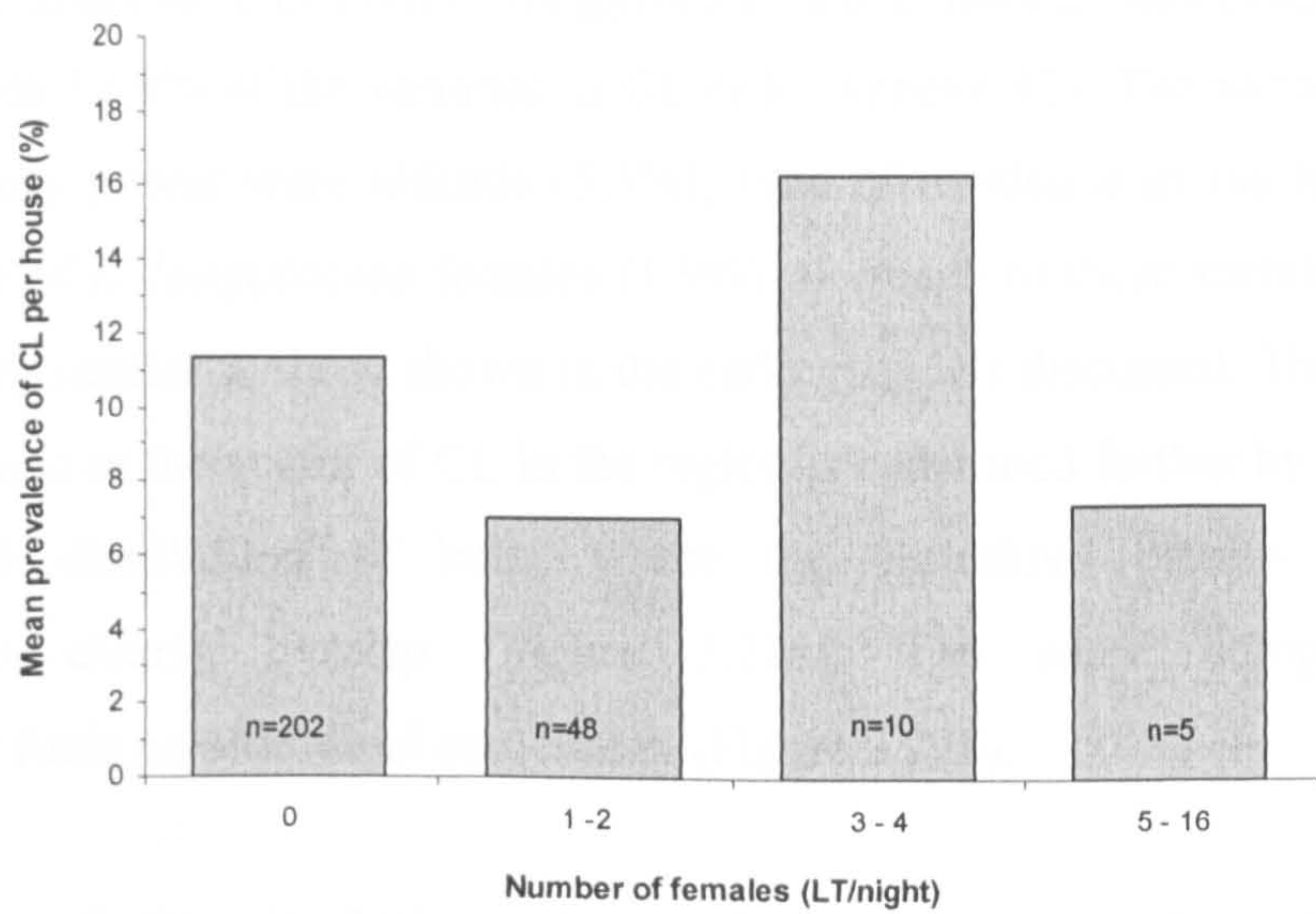


Figure 3.21 Mean cumulative prevalence of cutaneous leishmaniasis according to indoor abundance of *Lutzomyia nuneztovari* females (as measured by CDC light traps).

Finally, an analysis was carried out with all the 34 potential risk factor for CL prevalence plus the abundance of *L. longiflocosa* and *L. nuneztovari* in the Maximal Model. The results of this analysis confirmed the positive relationship between CL risk and *L. longiflocosa* and the negative relationship with *L. nuneztovari*. This final MAM

Table 3.9 Female abundance (ln [x + 1]) of *Lutzomyia longiflocosa* and *Lutzomyia nuneztovari* as risk factors for cutaneous leishmaniasis cumulative prevalence using logistic regression, and adjusting by length of residence in the house.

Explanatory variable	Odds ratio	χ^2	(df)	p	95% C.I.	
					Min	Max
<i>L. longiflocosa</i>	1.38	22.64	1	<0.001	1.11	1.73
<i>L. nuneztovari</i>	0.50	11.34	1	0.001	0.27	0.94
length of residence	1.03	26.92	1	<0.001	1.02	1.05

included the following significant variables: village, altitude, length of residence, gender and the abundance of both *L. longiflocosa* and *L. nuneztovari* (Table 3.10). The MAM explained 14.4% of the variance in CL risk (Annexe 42). The variables with the most explanatory power were altitude (5.5%), time of residence in the house (2.3%), and abundance of *L. longiflocosa* females (1.9%). For each of these variables, the odds ratios were very similar to those shown in the earlier models discussed. The pivotal role of *L. longiflocosa* as the vector of CL in the region is confirmed further by inspection of the altitudinal distribution of both, where the altitudinal pattern of CL and *L. longiflocosa* clearly overlap (Figure 3.22a). The same comparison with *L. nuneztovari* finds no altitudinal association (Figure 3.22b).

Reinforcement of the conclusions drawn from the statistical analyses of the relationships between sandfly abundance and CL prevalence is provided by inspection of the spatial distribution of the indoor abundance of *L. longiflocosa* and *L. nuneztovari* in relation to CL prevalence within each house (Annexes 43 - 48). *L. longiflocosa* has a widespread distribution in the three study villages, but with aggregated abundance in defined zones. These zones of high abundance are especially evident toward the North-East in La Troja village (Annexe 43) and toward the West in Brasilia village (Annexe 45). These zones overlap with the houses which presented the highest cumulative prevalence of CL in both villages. However, in the case of El Cedral village there is no

Table 3.10 Risk factors for cutaneous leishmaniasis cumulative prevalence, identified by logistic regression model incorporating sandfly abundance.

Explanatory variable		Odds ratio	S.E.	z	P> z	95% C.I.	
						Min	Max
Village	El Cedral	1					
	La Troja	2.00	0.643	2.15	0.032	1.063	3.754
	Brasilia	2.36	0.737	2.76	0.006	1.284	4.355
Altitude	1500 - 1599	1					
	1300 - 1399	0.68	0.688	-0.38	0.705	0.095	4.916
	1400 - 1499	0.42	0.152	-2.40	0.016	0.205	0.852
	1600 - 1699	0.38	0.135	-2.72	0.007	0.186	0.761
	1700 - 1799	0.20	0.074	-4.35	<0.001	0.095	0.411
	1801 - 1899	0.21	0.103	-3.17	0.002	0.078	0.549
	1900 - 2200	0.09	0.093	-2.29	0.022	0.011	0.702
Demographic features							
Length of residence (per year)		1.03	0.007	4.92	<0.001	1.020	1.048
Gender	Female	1					
	Male	1.41	0.247	1.97	0.049	1.002	1.987
Sandfly abundance							
<i>L. longiflocosa</i> females		1.38	0.144	3.10	0.002	1.126	1.695
<i>L. nuneztovari</i> females		0.47	0.114	-3.10	0.002	0.292	0.758

clear overlap between the high sandfly abundance zones and CL prevalence, maybe because the clumped pattern in this village is less evident (Annexe 47). In contrast,

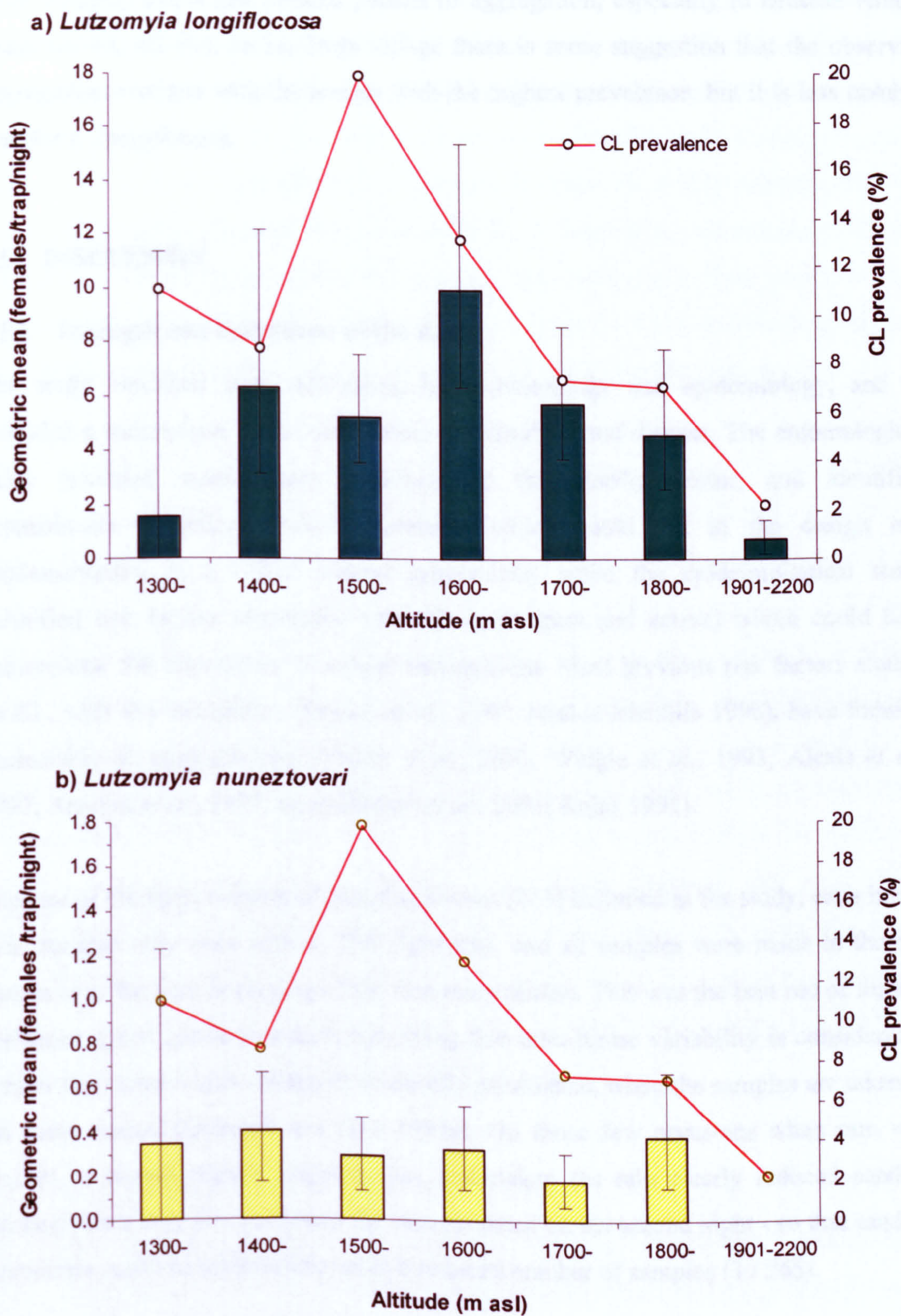


Figure 3.22 Cutaneous leishmaniasis cumulative prevalence and indoor female abundance (geometric means) of the two study sandflies by altitudinal ranges. Error bars are the 95% confidence intervals around the geometric means.

compared with *L. longiflocosa*, *L. nuneztovari* has a less widespread distribution (in all three villages) and a less defined pattern of aggregation, especially in Brasilia village (Annexes 44, 46, 48). In La Troja village there is some suggestion that the observed aggregation overlaps with the houses with the highest prevalence, but it is less notable than for *L. longiflocosa*.

3.5 DISCUSSION

3.5.1 Strength and limitations of the study

The study benefited from addressing both entomology and epidemiology, and so provided a comparison of the risk factors for sandflies and disease. The entomological study provided incriminatory evidence for the sandfly vector, and identified determinants of indoor vector abundance which could aid in the design and implementation of a vector control programme; while the epidemiological study identified risk factors associated with CL cases (past and active) which could help demonstrate the importance of indoor transmission. Most previous risk factors studies on CL, with few exceptions (Davies *et al.*, 1997; Muñoz-Mantilla 1998), have focused exclusively on epidemiology (Yadon *et al.*, 2003; Weigle *et al.*, 1993; Alcais *et al.*, 1997; Armijos *et al.*, 1997; Sosa-Eustani *et al.*, 2001; Rojas 1991).

Because of the large number of sampled houses (265) included in the study, each house was sampled only once with a CDC light trap, and all samples were made in the dry season or at the very beginning of the first rainy season. This was the best use of limited resources, given previous studies indicating that inter-house variability is considerably greater than intra-house variability in sandfly abundance, when the samples are taken in the same season (Quinnell and Dye 1994a). On those few occasions when rain was present, a second night's trapping was undertaken (as rain clearly reduced sandfly catches). On a very few occasions the rain persisted on the second night - so that sandfly abundance could be underestimated in a reduced number of samples (3 / 265).

The three villages studied were relatively close (maximum distance between villages of 40 km between La Troja and El Cedral), suggesting common features (such as relief, climate, and vegetation), and had similar sandfly communities (shown by the Morisita

index of similarity). But the study villages did demonstrate some differences in rainfall, land use, and socio-economical development, which could explain the differences detected in sandfly abundance and CL prevalence (see Chapter 2). For example, the percentage of land cover by forest and traditional coffee (within 300 m radius of each house) in La Troja, 23.5%, was almost twice that in Brasilia or El Cedral. However, inter-village (but not intra-village) differences in observed sandfly abundance could have been partially confounded by seasonality in *L. longiflocosa* abundance (which is apparently more abundant during dry seasons). The study took three months which involved one month of the first dry season (January) in La Troja and the beginning of the first rainy season in Brasilia (February) and El Cedral (March) (Annexe 24).

With this caveat, it appears that inter-village differences in CL risk are not fully explained by differences in sandfly numbers, as CL risk varied significantly with village even after adjusting for sandfly abundance. This could be because the entomological samples provided an insufficiently accurate measure of sandfly abundance, or because inter-village variation in CL risk is partly due to other factors, such as patterns of human exposure or the abundance of reservoir hosts. The main limitation of the epidemiology study was that it was cross-sectional. Hence, the current exposure to a putative risk factor might not have been the same at the time of infection. Fortunately, although there may be recall bias, it appears that the current exposure for most of the 34 tested risk factors (especially those factors related with house features and surrounding environment) have been reasonably stable during the last seven years, when 90% of the CL cases were reported. A second limitation is that the study only measured disease not infection. Not all infections cause disease, and heterogeneities in hosts susceptibility may have masked the impact of the risk factors measured.

3.5.2 Species composition and abundance indoors

All the species found indoors by CDC light traps, with the exception of *L. trinidadensis* and *L. dubitans*, were also collected by human landing either outdoor (*L. longiflocosa*, *L. nuneztovari*, *L. columbiana*, *L. erwindonaldi*, *L. oresbia*, *L. (Helcocyrtomyia) spp.*) (Chapter 2) or indoor (*L. longiflocosa*, *L. nuneztovari*, *L. columbiana*) (Chapter 4) during the other studies carried out in this project. Further evidence for endophagy and anthropophagy for the main species, *L. longiflocosa*, comes from the intervention trial

(Chapter 4) during which 95% of all blood meals identified from wild caught females (with fresh blood) from 16 control houses were from humans. While the other species collected were relatively infrequent, it remains feasible that their relative abundance could change during the rainy seasons. With this caveat, it appears that a single anthropophilic and synanthropic species (*L. longiflocosa*) is overwhelmingly dominant both indoors (93.5%) and outdoors (see Chapter 2). Such single species dominance is a feature of many studied CL foci in the sub-Andean region (e.g. Cárdenas *et al.* (1999); Ferro *et al.* (1999); Montoya-Lerma *et al.* (1999); Velez *et al.* (1991); Alexander *et al.* (1995d); Valenta (1999); Torres *et al.* (1998); Martinez *et al.* (1999); Davies *et al.* (1997)). Outside sub-Andean region the pattern could be different, for instances in the foothills where the dominance of the most abundant species is low (e.g. Muñoz-Mantilla 1998).

3.5.3 Vector incrimination

The statistical and biological evidence gathering during the present study strongly point to *L. longiflocosa* as the only important vector of CL in the sub-Andean region of Huila department. The statistical evidence is the consistent demonstration of a highly significant positive association between the indoor abundance of *L. longiflocosa* females and CL cumulative prevalence. This strongly indicates a significant degree of transmission in the domestic environment and is consistent with the primacy of indoor transmission. In contrast, the lack of positive association of *L. nuneztovari* with CL cumulative prevalence indicates that this species has no significant vector role in this region, at least in the intra-domiciliary environment of the study area. The negative association of *L. nuneztovari* with CL when both sandflies species were included in the model, could be explained by the apparent antagonism between them – i.e. their different ecological requirements (see Chapter 2).

Further “biological” evidence in support of the principal vectorial role for *L. longiflocosa* (as compared with *L. nuneztovari*) comes from four sources: (1) overwhelming indoor abundance of *L. longiflocosa* (93.5% of the 7659 sandflies caught in 265 houses), and low abundance of *L. nuneztovari* (2.1%); (2) the wider distribution within houses of *L. longiflocosa* (85.7% of all houses were positive for *L. longiflocosa*) and lower of *L. nuneztovari* (26.8%); (3) the apparent association

between the altitudinal distribution of CL and that of *L. longiflocosa* (with peak altitudes at 1500 m a.s.l. and 1600 m a.s.l., respectively), while *L. nuneztovari* was not related; and (4) in at least two of the three study villages, overlapping of the zones with the highest indoor abundance of *L. longiflocosa* (but not *L. nuneztovari*) and the zones with the highest household CL prevalence.

Regarding the spatial distribution of *L. longiflocosa*, the results suggest that most people are exposed to infection with *Le. braziliensis* but at very different rates (assuming the abundance of all female sandflies correlates with the abundance of infected females): *L. longiflocosa* was present in nearly all houses (86%), but highly aggregated, with 80% in only 16% of houses. This pattern is similar to that reported for *L. longipalpis* vector of *Le. chagasi* in Marajo island, Brasil, where 85% of sandflies were caught in 9% of the houses (Quinnell and Dye 1994a). This heterogeneity in human-vector contact has been shown to be an important determinant of the epidemiology of vector-borne diseases; and has been immortalised by the term, “the 20 / 80 rule”, indicating that 20% of a host population typically contribute at least 80% of the net transmission potential (Woolhouse *et al.*, 1997). In terms of possible control for CL in the study area this implies that a control program targeting those houses with the highest abundance of *L. longiflocosa* (e.g. 15.8%) would be highly effective. However, the problem is of course identifying those high abundance houses. One approach is to look for easily observable risk factors which correlate strongly with high sandfly abundance. The results of this chapter suggest that there are risk factors (see below), but it is unclear whether their predictive power is sufficient, as yet, for targeting control activity.

3.5.4 Risk factors

The CL cumulative prevalence (scars and lesions) found in this study, 11.4% (162 / 1,420) largely reflects the recent epidemic in the sub-Andean region of Huila department: from 1993 to 1996. This indicates that during the epidemic the average annual incidence rate was approximately 3% per year. Although only 58% of cases detected in this study were detected in the SSDH records, the gender and age distribution of the detected cases were surprisingly similar to that described by the SSDH records (1262 cases from 1982 – 1995). Both data bases found: (1) a clear male bias amongst adults, 1.7:1 (male:female); (2) no clear gender bias amongst children,

1.3:1 (Tables 3.7 and 1.4); and (3) a relatively high percentage of cases amongst children: for females, 36.7% (22 / 60), in this study (0 - 11 years old) and 31.4% (135 / 430), in the SSDH records (0 - 10 years old); and for males 28.4% (29 / 102) and 24.3% (178 / 732), respectively. So, it seems that activities specific to adult males are a risk factor for CL within the study area, but that domestic transmission (which puts the whole family at risk) is probably the main cause of CL in this region, as in several other CL foci in the Andean region (e.g. Muñoz-Mantilla (1998); Torres *et al.*, (1989); Davies *et al.*, (1997); Hashigushi *et al.*, (1990); Velez *et al.*, (1987)). This would explain the similar CL prevalence in the youngest children, up to 3 years old, 9.2%, compared with the other age-groups (Figure. 3.18).

The apparent reduction in CL prevalence in elderly people detected in this study also requires comment (Figure 3.18). This pattern could be explained by: (1) recall bias, as people who had been infected in childhood may not have complete recall of their disease status, and the scars may be harder to detect; or (2) immunity, as these people may have developed immunity following sub-clinical infections.

After adjusting for village differences, altitudinal variation between houses was the risk factor with the greatest explanatory power for indoor abundance of *L. longiflocosa* females (but not *L. nuneztovari*). Peak female *L. longiflocosa* abundance was detected in houses between 1600 - 1699 m a.s.l. , and there was a strong reduction in abundance in houses above 2000 m a.s.l., suggesting that this is close to the altitudinal upper tolerance limit for *L. longiflocosa*. The results of this household comparison study are consistent with the geographical study described in Chapter 2, in which *L. longiflocosa* abundance peaked between 1500 - 1699 m a.s.l. and the lower limit of tolerance was between 900-1000 m a.s.l. ; but disagree about the upper limit of tolerance which in the latter study was not defined because *L. longiflocosa* abundance outdoors between 2000 - 2200 m a.s.l. was relatively high.

Inter-house variability in altitude was also the most important risk factor, for CL risk, even after adjusting for variability in sandfly numbers. CL risk peaked between 1500 - 1600 m a.s.l., relatively close to the optimum altitudinal range for indoor *L. longiflocosa* abundance (1600 - 1699 m a.s.l.). It should also be noted that most of the human population are concentrated, 53% (755 / 1,427), between 1500 - 1699 m a.s.l. . Hence,

the intolerance zones apparently detected for *L. longiflocosa*, suggest the risk for CL caused by *L. longiflocosa* in Huila department is negligible below 900 m a.s.l. or above 2200 m a.s.l. (limit pending to be confirmed).

Our inability to detect any association between house “features” and indoor abundance of *L. longiflocosa* was unexpected because house variability should affect accessibility or attraction to sandflies. For instance, increasing area of house openings has been associated with increasing indoor abundance of *L. longipalpis* (Quinnell and Dye 1994a) and an increasing proportion of *L. whitmani* entering a house (D. Campbell-Lendrum, personal communication). For both species, this pattern was also demonstrated by comparing sandfly populations entering experimental chicken sheds with varying degrees of wall closure (Quinnell and Dye 1994b; Campbell-Lendrum 2000). Roofs made of thatch also increased the abundance of *L. longipalpis* (Quinnell and Dye 1994a); while mud walls and divided rooms were associated with a reduction in *L. whitmani* abundance (D. Campbell-Lendrum, personal communication).

In the present study, roof type was not included in the analysis as all houses had roofs made of corrugated zinc. Similarly, electricity service (time since installed) was also excluded as a predictor for *L. longiflocosa* abundance. Based on the known phototropism of this species (which is highly attracted to CDC light traps) this was unexpected, but could be explained by the fact that most of the houses had this service (89.5%), with little variations in the period of time since installed (9.1 years, C.I. 8.2 - 10).

Our inability to detect any association with openings may be explained by the relatively large openings presented in nearly all the houses, average 5.8 m² (C.I. 5.0 - 6.7 m²). Hence, all the houses in this study site may be above the upper limit where openings had any effect in reducing sandfly access to the houses. This hypothesis has support from the large house comparison study carried out in Brazil by Campbell-Lendrum *et al.* (personal communication). From three sites across Brazil where openings were tested as predictors of the proportion of *L. whitmani* entering houses, with mean openings per house of 0.2 m², 1.1 m² and 3 m², this variable was only significant in the site with the lowest mean openings. Additional evidence that variation in openings impacts on sandfly access across a small range comes indirectly from some CL risk

factor studies. In Santiago del Estero, Argentina the presence of "holes instead of windows" increased the risk for CL (odds ratio: 8.0, C.I.: 2.5 - 26) (Yadon *et al.*, 2003). In Salta, also in Argentina, the presence of cracks on windows, apparently, increased the risk of infection for CL (odds ratio: 2.9, C.I.: 0.88 - 9.7), although only by univariate analysis (Sosa-Eustani *et al.*, 2001). In Piura, Peru, holes in bedroom windows was identified as potential risk (odds ratio: 6.3, C.I.: 0.75 - 53.6) (Llanos-Cuentas 1994). Hence, some sandfly species are clearly able to enter houses even when only relatively small openings are available. Accessibility of houses with large openings is relatively insensitive to variability in opening size. These findings could be of importance when designing a vector control programme using impregnated curtains to prevent sandfly access to houses.

The relatively high abundance of *L. nuneztovari* in houses with a "close plank" ceiling was unexpected. Sandfly access (and hence abundance) was expected to be greater for houses with ceilings with larger openings (i.e. "close plank and hole" or "plank with spaces" or "no ceiling"). If the result is not just a statistical aberration, one explanation could be that "close plank" ceilings help to maintain constant and favourable microclimatic conditions for sandflies. In contrast, houses without ceilings (which all have roofs made of corrugated zinc) present extreme changes in temperature, being very hot during the day and very cold during the night. A similar explanation was suggested for the positive association between *L. longipalpis* and thatched roofs in Marajo island (Quinnell and Dye 1994a). However, it is unclear why this factor is important for *L. nuneztovari* but not for *L. longiflocosa*.

The relatively low CL risk for people living in "huts" was unexpected as this type of house has a range of features generally believed to be risk factors for CL instead of being protective: for instance, roofs made of thatch (Quinnell and Dye 1994a; Weigle *et al.*, 1993), floor type made of soil-earth (Yadon *et al.*, 2003; Llanos-Cuentas 1994), and walls made of wood/cane (Armijos *et al.*, 1997). The most plausible explanation is that this is a chance result, as the effect has only borderline significance and poor explanatory power.

The positive relationship between the numbers of persons per house and the indoor abundance of female *L. longiflocosa* is explained probably by the increasing

attractiveness to sandflies of an odour plume with an increasing concentration of human kairomones. This hypothesis is supported by experimental evidence showing that attractiveness to *L. intermedia* and *L. whitmani* increases in direct association with CO₂ dose (representing equivalents to the CO₂ release by 0.5, 1, 2, and 4 persons) (Pinto *et al.*, 2001). Experimental field studies have also shown that the order of host preference for *L. whitmani* (Campbell-Lendrum *et al.*, 1999), *L. longipalpis* (Quinnell *et al.*, 1992), and *L. evansi* (Montoya-Lerma and Lane 1996) is a function of host density or size.

The highly significant negative association between indoor abundance of *L. longiflocosa* females and the number of dogs within 200 m of the house suggests that dogs could have a protective effect for CL. In the study villages dogs usually sleep outside but close to the houses (at a mean distance of 3.5 m). They could therefore divert sandflies from entering houses by acting as a more accessible blood source than humans sleeping indoors. Negative associations between peridomestic domestic animals and indoor sandfly abundance has been recognised in at least one previous study (D. Campbell-Lendrum, personal communication) in Brazil, where the ratio of indoor: outdoor *L. whitmani* females dropped with the number of fowl within 50 m of house. On the other hand, contrasting results were observed by Quinnell and Dye (Quinnell and Dye 1994a) who reported that indoor abundance of *L. longipalpis* increased with dog ownership, indicating that dogs were attracting more sandflies into the vicinity of the houses. One can only speculate on the reasons for these different findings, but the effect of particular peridomestic animals on indoor sandfly abundance is clearly dependent on sandfly host preference, sandfly ecology, sandfly endophagy, the relative frequency of different host types available in the domestic environment, and the relative “openness” of houses. Where sandfly populations in the domestic environment are not greatly enhanced by the presence of dogs, diversion of sandflies to dogs could reduce indoor sandfly abundance; whereas where sandfly population densities are low, dogs may act as a significant attractant towards dwellings, and so cause the opposite effect. It should be noted that the attractiveness of dogs for *L. longiflocosa* has not yet been tested.

The indoor abundance of *L. nuneztovari* had the same type of relationships with humans and dogs as did *L. longiflocosa*; and additionally showed a negative association with the presence of pigs. The explanation for these results is presumably as for *L. longiflocosa*. It should be pointed out that the relation between *L. nuneztovari* with the number of

persons per house was weak compared with *L. longiflocosa*, suggesting the *L. nuneztovari* is less anthropophilic.

Given the associations detected with indoor sandfly abundance, it was surprising that no association was detected between host distributions and CL cumulative prevalence. The most likely explanation is that the status of host populations (humans, dogs, pigs) variables has not been stable over time, and so the current measurements have little explanatory power for the risk of CL in past years. An additional explanation could be that not all CL cases were caused by transmission in the domestic environment – so diluting the observed effect of any house feature on CL risk (despite its observed impact on indoor sandfly abundance)

The negative association between grass and the indoor abundance of *L. longiflocosa* could be explained by a spatial barrier or "buffer zone", assuming that pasture is an unsuitable habitat for sandflies to breed or rest in. Land covered by grass around houses increases the distance which sandflies have to travel from the forest or a similar habitat (e.g. traditional coffee) to the domestic environment in search for hosts, and also could be an unsuitable area because of the potentially high wind speed. Open areas, such as pasture, has been previously associated as habitats of low abundance for some sandfly species in the sub-Andean region. In the El Opon study, a multivariate analysis including 35 possible sandfly-vegetation associations allowed found a negative correlation between the percentage of land cover by pasture (within 50 m of a house), and the indoor abundance of *L. trapidoi* (Muñoz-Mantilla 1998). Similarly, in Mérida, Venezuela, the abundance of *L. youngi* and *L. nuneztovari* was significantly lower in "open grass" compared with forest, secondary forest and coffee plantation (presumably "traditional coffee") (Valenta 1999). Outside the Andean region, in Marajó island, Brazil, the first quantitative comprehensive study on sandfly risk factors at household level, the abundance of *L. longipalpis*, in sheds, was found significantly lower in savanna habitat compared with cultivated areas or open woodlands (Quinnell and Dye 1994a).

The apparent protective effect of grasslands (due to deforestation) could have implications for CL control strategies. Notably, WHO (1990) suggested that clearing vegetation around houses and the creation of forest free zones could be used as control

measures. Taking into account that in the sub-Andean region the estimated range of dispersion for many sandfly species is below 200 m (Alexander and Young 1992), maybe an area of 300 m radius around each house would be needed as a "buffer zone". As the average distance between houses within this study area was 147 m, this would mean practically the total destruction of the remaining forest. Such a measure is not practical or to be recommended as: (1) these forests though already deteriorated have highly diverse fauna and flora (in the all Andes only 25% of the primary vegetation has remained), and belong to one of two "biodiversity hyper hotspots" which are prioritised for conservation (Myers *et al.*, 2000); and (2) further deforestation would interfere with the water cycle as the forest remnants protect sources of streams, rivers or areas with threat of erosion.

An alternative policy would be to clear only a smaller area around houses, but this is less likely to reduce indoor sandfly abundance. Sandfly populations can persist in relatively small forest (or traditional coffee) refugia. Alexander *et al.* suggested that 2 ha of "traditional coffee" (an equivalent to 7% of the land cover within a 300 m radius of a house) in the Colombian Andean foothills is sufficient to maintain a community of 17 sandflies species, including 11 anthropophilic species, two suspected CL vectors (*L. ovallesi* and *L. gomezi*), and one confirmed CL vector (*L. spinicrassa*) (Alexander *et al.*, 1992, 2001).

The positive association detected between indoor abundance of *L. nuneztovari* and the abundance of banana plants within 50 m of a house could be explained by the sandfly requirement for sugar meals (No such association with bananas was detected for *L. longiflocosa*, suggesting some inter-specific differences in sugar feeding behaviour). Sugar increases sandfly survival and oviposition rates, and is acquired either by direct piercing and sucking of plant tissue (Sneider and Warburg 1986) or from secretions of aphids and coccids (Killick-Kendrick and Killick-Kendrick 1987; Hamilton and Elnaïem 2000). In a study of the sugar sources of sandflies in Colombian coffee plantations, banana (plantain) plants, *Musa paradisiaca*, were not found to be a sugar source for *L. youngi*, but there was evidence that *L. youngi* fed on honeydew produce by aphids (*Pentalonia sp.*) who lived on this plant (Alexander and Usma 1994). So it is possible that aphids living on banana plants in the study area are a source of sugar for *L. nuneztovari*. However, the presence of aphids was not confirmed. In other sites of the

sub-Andean region, an association has been detected between sandflies and plants who harbour aphids (for *L. verrucarum* in Perú) (Cameron *et al.*, 1994).

The lack of association between forest and/or "traditional coffee plantations" with sandfly indoor abundance was unexpected. One explanation could be that the sampled forests and "traditional coffee" were heterogeneous in relation to the characteristics influencing sandfly survival, as illustrated by the detection of ten distinct habitats types into which these two main habitats were divided using the physiognomic-structural classification (Chapter 2, section 2.3.4.2). Another possible explanation for the lack of association between forest and sandfly indoor abundance could have been that the forest refugia were too small to harbour sandfly populations. This possibility is deemed unlikely because the mean percentage of land around houses covered by forest in this study (14.1%) is approximately 4.0 ha, which is twice the area previously mentioned as sufficient for maintaining a diverse sandfly population (Alexander *et al.*, 1992). On the other hand, "traditional coffee" was relatively rare in the study area – present around only 12.6 % of houses – and so the sample was probably insufficient to test reliably its impact on indoor sandfly abundance. Analyses where forest and "traditional coffee" were merged in one type of vegetation (not shown) also failed to find any association.

The only published study which explicitly measures the impact of the extent of an arboreal habitat on indoor sandfly abundance is the study carried out in El Opon, where the percentage of land covered by cacao plantation (a shaded crop) within 300 m, 100 m and 800 m of the houses, was positively correlated with the indoor abundance of *L. trapidoi*, *L. gomezi* and *L. ovallesi*, respectively (Muñoz-Mantilla 1998).

The negative association between the indoor abundance of *L. longiflocosa* and the number of houses within 100 m could be due to the impact of house proximity on surrounding habitat. Secluded houses with one house or none within 100 m, as compared with 2 or more, tend to be surrounded by more forest and by more "traditional coffee" but by less "intensive unshaded coffee". However, unexpectedly, "secluded" houses tend to be surrounded by more grass.

A second speculative explanation for the observed pattern could be that sandfly populations in this region are largely dependent on the extent of forest and traditional

coffee (where they presumably breed), and not on houses. Hence, when houses are close to each other they are essentially “competing” to attract the same sandflies; whereas a secluded house has a relatively large sandfly population to draw from without competition.

As for the analyses of host abundance described above, given the associations detected between habitat and indoor sandfly abundance, it was surprising that no association was detected with CL cumulative prevalence. As for hosts, one possible explanation is that the habitat has not been stable over time (as deforestation has been rife), and so the current measurements have little explanatory power for the risk of CL in past years. Another explanation is that not all CL transmission takes place in the domestic environment.

Detailed description of the control measures of control is presented in Chapter 5. However, it is notable that none of the recorded measures to control insect bites practiced by households in the study area (smoke, spraying with insecticides and non-insecticidal substances, and use of bednets) was associated with the indoor abundance of *L. longiflocosa* or *L. nuneztovari* or with CL cumulative prevalence. There are several possible explanations for this result: (1) most of the control measures seem to have short term effect; (2) the measures were used temporally, only when the sandflies become a nuisance for the householders during the season of high abundance (dry months); and (3) the protective impact of insect control measures is counterbalanced by the fact that control measures are more likely to be used by householders who experience greater number of insect bites.

Conclusions: What is the vector and where does most transmission occur?

The results suggest that *L. longiflocosa* is the main vector of CL in Huila department, at least during the last epidemic (1993 - 1996). In Huila as in other Andean regions, sandfly species tend to be restricted to specific environments defined by their altitudinal ranges, and CL risk is therefore closely associated with altitude. Also, as in other Andean regions, a major proportion (but not all) of CL transmission in Huila department occurs indoors. The main finding which supports indoor transmission is the positive association between indoor abundance of *L. longiflocosa* and household cumulative prevalence of CL. Transmission away from houses is supported by the apparent increase

in CL risk for adult males. Unfortunately, the risk factor study focused largely on features likely to impact on indoor transmission, and no data were collected on human activities which could have increased occupational exposure. These variables should be tested in future to provide an unbiased comparison of the risks of exposure in different sites of CL transmission.

Finally, the association between *L. longiflocosa* indoors abundance and CL risk indicates that reducing the rate of sandfly bites inside houses could reduce the incidence of CL in Huila department. As there is no evidence for any endophilic behaviour in the study area, insecticide treated nets could be a more suitable control measure than residual insecticide spraying of houses. On the other hand, the analysis of the effect of house features leaves open the possibility that house improvements could reduce CL risk. All houses had such large openings that the observed variation in opening size had no measurable impact on indoor sandfly abundance. Hence, a reduction in house openings, though logistically difficult to implement, might be effective.

4 SANDFLY CONTROL

4.1 INTRODUCTION

Sandfly control campaigns by the Health Services in Huila department generally follow the pattern for the sub-Andean region, this involves sporadic house spraying with residual insecticide (indoors and outside houses) during outbreak periods without any evaluation or monitoring. In 1999, an alternative strategy was employed (but again not evaluated or monitored) by the Health Services, namely the provision of 1000 insecticide treated bednets (ITNs) in six villages (220 houses) of one of the epidemic municipalities. The studies described in this chapter aim to provide the evidence basis for deciding which, if any, of these two strategies is the most appropriate for controlling sandfly transmission of cutaneous leishmaniasis in this region.

4.1.1 House spraying with insecticides

In global terms, house spraying with residual insecticides has been the control measure most used, being recommended by the WHO (1990) against endophilic species, i.e. species which tend to rest in man-made constructions for a whole or a defined part of the gonotrophic cycle (Clements 1999). For instance, in Brazil, house spraying is a major tool for VL control by the Ministry of Health with ca. 56,000 houses/year sprayed for this purpose with DDT or deltamethrin (Lacerda 1994).

Effectiveness of house spraying has been claimed as a by-product of malaria eradication programs, mostly by circumstantial evidence. For instance, Vioukov (1987) mentioned how, in India, kala-azar (VL) incidence was reduced during and after the malaria control campaign of the 50s and 60s. Similar reductions in leishmaniasis were attributed to malaria control in Pakistan. However, in Greece malaria control significantly reduced the incidence of sandfly fever but had no impact on VL; and in Iraq anthroponotic CL declined (as in the Central Asian republics and Transcaucasus) but no reduction was observed in VL. In Peru there is strong evidence that the use of DDT in the anti-malaria campaign from the 1950s to the 1970s resulted in a transient reduction of CL cases

(Davies *et al.*, 1994), but in Venezuela, malaria control had no detectable side effects on leishmaniasis (Desjeux, 2001).

The apparently contradictory results of the collateral effects of malaria control programmes on leishmaniasis are presumably due to differences in the ecology of the vectors and in the epidemiology of the different leishmaniasis. In particular, leishmaniasis control was achieved where sandfly vectors were strongly endophilic and an important part of transmission took place indoors.

Direct measurements of the impact of house spraying on CL transmission come from only a few published trials (summarised in Table 4.1). An additional study (Reyburn *et al.*, 2000) which compared house spraying with other treatments including bednets is cited in Table 4.2. With few exception (Davies *et al.*, 2000a; Reyburn *et al.*, 2000), most of these trials do not provide incontrovertible evidence (either epidemiological or entomological) for the effectiveness of house spraying. The main reasons for this are: (1) the absence of replicates (Le Pont *et al.*, 1989c; Benzerroug *et al.*, 1992; Falcão *et al.*, 1991); (2) the small sample size of the trial (Alexander *et al.*, 1995a; Falcão *et al.*, 1991); or (3) the absence of contemporaneous controls (Benzerroug *et al.*, 1992). Nevertheless, the results of these trials indicate that house spraying, as expected, is specially effective to control endophilic sandflies species, such as *Lutzomyia verrucarum*, *L. peruensis* (Davies *et al.*, 2000a) and *L. intermedia* (Falcão *et al.*, 1991), in the New World; and *Phlebotomus papatasi* (Benzerroug *et al.*, 1992) in the Old World. In contrast, house spraying failed to reduce the abundance of exophilic sandflies, such as *L. nuneztovari* in Bolivia (Le Pont *et al.*, 1989c), which have relatively little probability of contact with the treated surfaces (walls and ceilings).

The main observed effects of house spraying in field trials are a reduction in indoor sandfly abundance (Davies *et al.*, 2000a; Falcão *et al.*, 1991; Le Pont *et al.*, 1989c) and a reduction in blood-fed females collected in light traps (Davies *et al.*, 2000a; Le Pont *et al.*, 1989c). However, the latter may reflect trapping bias in sprayed houses rather than a real inhibition of indoor bloodfeeding due to the treatment. It should be noted that all village-based intervention trials (Falcão *et al.*, 1991; Benzerroug *et al.*, 1992)

Table 4.1 Summary of recent field trials on house spraying for sandflies and cutaneous leishmaniasis control.

Country and locality (Reference)	Vector species	Insecticide (Dose)	Type of treatment	Study design (Sampled unit; sample size and treatments ^a)	Outcomes (Sampled technique)	Evaluation frequency (Duration)	Results
Peru, Lima, Ancash and Piura (Davies et al., 2000a)	<i>L. verrucarum</i> <i>L. peruensis</i>	Lambda-cyhalothrin CS (25 mg/m ²)	Indoor adobe walls sprayed at 6 months intervals.	Matched randomized study. (Household; 112 HSP, 154 CON)	Ent: Sandfly indoors abundance (CDC light traps).	Ent: 16 times. 1 pre-int and 15 pos-int (2 years).	Ent: <i>L. verrucarum</i> : 78% reduction in abundance. <i>L. peruensis</i> : 83% reduction in abundance. 77% reduction in proportion of bloodfed sandflies (comparison post-int only).
				Ent: Sub-sample of houses (22 HSP, 21 CON) which were matched based on pre- intervention sandfly data. Comparison pre- int vs. pos-int and pos-int. Bioassays to confirm residual effect. Epi: Houses in th small hamlets were matched based on pre-intervention Epi data. Population with not prior history of infectious or disease: 196 persons in HSP, 241 persons in CON. Comparison pos-int.	Epi: CL incidence (leishmanin skin test, interview survey).	Epi: 2 times. 1 pre-int and 1 pos-int (2 years).	Epi: Reduction in incidence from 10% (24/241) in the CON to 4.6% (9/196) in the HSP treatmEnt Incidence reduction was 65%, excluding cases of the first 3 months pos-int.
Brazil, Espirito Santo State (Falcao et al., 1991)	<i>L. intermedia</i>	Deltamethrin (25 mg/m ²)	Indoors, annexes and trees 10 m around the house.	Not randomized study. (Village; 1 HSP, 1 CON; no replicates) Ent: Pre-int: random sampling of sub- sample of houses (9 HSP, 9 CON). Pos- int: Random sampling in sub-sample of houses (25) in CON village and all houses in the HSP village. Comparison pre-int vs. pos-int and pos-int. Bioassays to confirm insecticidal power.	Ent: Sandfly indoors and outdoors abundance (Falcao's light traps).	Ent: 6 times. 1 pre-int and 5 pos-int (1 year).	Ent: Reduction of indoors sandflies abundance pos-int in 5 time points (0.4 - 1 s/t/n) in the HSP village compared with the single time point in the same village pre-int (8 s/t/n), and in 2 of 5 point post-int compared with the CON village. Not reduction in sandfly abundance around houses.
				Comparison pre-int and post-int. (Village; 1 pre-int, 1 pos-int, no replicates) Ent: Comparison pre-int vs. pos-int in a village with 70 houses. Pre-int: Sub- sample of 50 houses and 7 chicken sheds. Pos-int: Sub-sample of 14-17 houses and 3 chicken sheds. Sampling based on location and compliance of households. Data on control area were taken but not presented because they did not correspond as expected.	Ent: Sandflies abundance indoors and outdoors (CDC light traps).	Ent: 12 times. 1 pre-int and 11 pos-int (1 year).	Ent: <i>L. nuneztovari</i> : Not effect on abundance, but indoors blood fed- females was reduced significantly (>50%). <i>L. longipalpis</i> : Reduction in indoors abundance and almost elimination in chicken sheds.
Bolivia, Las Yungas (Le Pont et al., 1989c)	<i>L. nuneztovari</i> <i>L. longipalpis</i>	Deltamethrin (25 mg/m ²)	Indoors, annexes and trees around the house.	Comparison pre-int and post-int. (Village; 1 pre-int, 1 pos-int, no replicates) Ent: Comparison pre-int vs. pos-int in a village with 70 houses. Pre-int: Sub- sample of 50 houses and 7 chicken sheds. Pos-int: Sub-sample of 14-17 houses and 3 chicken sheds. Sampling based on location and compliance of households. Data on control area were taken but not presented because they did not correspond as expected.	Ent: Sandflies abundance indoors and outdoors (CDC light traps).	Ent: 12 times. 1 pre-int and 11 pos-int (1 year).	Ent: <i>L. nuneztovari</i> : Not effect on abundance, but indoors blood fed- females was reduced significantly (>50%). <i>L. longipalpis</i> : Reduction in indoors abundance and almost elimination in chicken sheds.

Table 4.1 continued.

Country and locality (Reference)	Vector species	Insecticide (Dose)	Type of treatment	Study design (Sampled unit; sample size and treatments ^a)	Outcomes (Sampled technique)	Evaluation frequency (Duration)	Results
Algeria, M'sila (Benzerroug et al., 1992)	<i>P. papatasi</i>	DDT WP (1900 mg/m ²)	Indoors and around houses.	<u>Ent.</u> Not randomized study. (Village; 1 HSP, 1 CON, no replicates) Not details on number of sampled houses. Sampled area around 1.6 m ² / treatment / sampled point. Comparison pos-int. Higher abundance of sandflies pre-int in the HSP village.	<u>Ent.</u> Sandfly abundance inside (?) houses. (Sticky traps). <u>Epi.</u> CL incidence (case notification and interview survey).	<u>Ent.</u> 6 times (6 months) and once (>2 years). <u>Epi.</u> 3 times (3 years).	<u>Ent.</u> Sandfly reduction: 11 s/m ² to 171 s/m ² in the CON village compared with 2.5 s/m ² to 30 s/m ² in the HSP village. <u>Epi.</u> Overall reduction from 426 CL cases x100.000 pre-int to 17.9 cases x100.000 pos-int.
				<u>Epi.</u> Comparison pre-int and post-int. (Village; 1 pre-int, 1 pos-int, no replicates) Comparison pre-int vs. pos-int. No control area. Around 18.000 houses included.			
Colombia, Valle del Cauca (Alexander et al., 1995a)	<i>L. youngi</i> <i>L. columbiana</i>	Deltamethrin, WP (500 mg/m ²)	Indoors	Matched randomized (household; 6 HSP, 6 CON) <u>Ent.</u> Houses matched by house features and surrounding habitat. For sandfly abundance all houses were sampled, while a sub-sampled of houses (3 HSP, 3 CON) were taken for human biting. Comparison pos-int. No control pre-int. Bioassays to confirm insecticidal power.	<u>Ent.</u> Indoor abundance (sticky traps). Indoor Human biting rate (human landing).	<u>Ent.</u> Weakly (6 months).	<u>Ent.</u> <i>L. youngi</i> : Abundance by sticky traps higher in HSP houses compared with the CON houses. Human landing with not significant differences. <i>L. columbiana</i> : Not significant differences for any of the two sampling methods.

^a Treatments: HSP: House spraying, CON: Control, without any intervention; Ent: Entomological; Epi: Epidemiological; Pre-int: Pre-intervention; Pos-int: Post-intervention; (?) Uncertain.

(Le Pont *et al.*, 1989c) failed to detect any reduction in sandfly population (i.e. mass killing effect). However two household-based intervention trials showed that house spraying significantly reduced CL incidence. In the New World, ZCL incidence was reduced from 10% (24 / 241) in control houses to 4.6% (9 / 196) in sprayed houses (Davies *et al.*, 2000a); and in the Old World, the incidence of anthroponotic cutaneous leishmaniasis (ACL) was reduced from 7.2% (92 / 1281) in control houses to 4.4% (36 / 813) in sprayed houses (Reyburn *et al.*, 2000).

Perhaps the most influential insecticide spraying trial in relation to leishmaniasis control was the study of Alencar (1961) in Ceará state, Brazil, describing the impact on VL. The study compared VL incidence during eight years in 14 DDT (1500 mg/m²) sprayed municipalities with equal number of control municipalities. The results apparently showed that in the sprayed municipalities VL cases during the last 4 years were reduced by 58%, compared to an increase of 10% in the control villages. However, this study was flawed as the spraying was not continuous during the study period (most of municipalities were sprayed once) and the comparison of the two group of years for the sprayed municipalities was biased because the "pre-intervention" group included several treated municipalities. Nevertheless the results were used as the primary evidence to support the longstanding national house spraying campaign against VL in Brazil.

4.1.2 Insecticide treated bednets

In recent years the use of insecticide treated bednets (ITNs) for vector control has become a useful alternative to house spraying, especially for malaria. Notably, in Africa the use of ITNs has resulted in a significant reduction in child mortality as well as in disease incidence (Lengeler *et al.*, 1996). In Colombia too, preliminary trials indicated that ITNs alone (Kroeger *et al.*, 1995) or combined with others measures of control (Rojas *et al.*, 1992) can cause a reduction in malaria. Insecticide treated bednets can be considered as baited traps where vectors pick up a lethal dose of insecticide when they alight on the bednet. So, compared with house spraying, this measure has the following advantages: a) their effectiveness is expected to be independent of the endophilic or exophilic behavior of the vectors; b) less insecticide is used; c) there is participation of the community in the control, so there is no strong dependence on a vertical control programme. For safety and efficacy reasons, the insecticides used to treat bednets are

synthetic pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin). These are neurotoxic contact insecticides, affecting the central and peripheral nervous systems of insects. In the peripheral system pyrethroids affect the sodium channels changing the action potential of neuronal membranes, causing an over excitation which blocks sign conduction. As a result the knockdown effect is produced, followed in most cases by death of the insect.

Most studies on the impact of pyrethroid treated bednets on bloodsucking insect behaviour have focused on mosquitoes entering experimental huts trials. The main effects (Lines 1996) are the following: (1) Deterrency. Fewer mosquitoes enter the bedroom. Given the low vapour pressure of pyrethroids, this effect is presumably mediated by insecticide-contaminated dust producing an apparent airborne effect. (2) Feeding inhibition. A lower proportion of mosquitoes which enter the bedroom feed. (3) Mortality. A proportion of mosquitoes die before or after biting. This could lead to a mass-killing effect if a sufficiently high proportion of houses use ITNs (4) Excito-repellency. After contact with a treated surface, mosquitoes are stimulated to leave (Miller *et al.*, 1991) rather than feed on unprotected hosts in the same room as hosts sleeping under an ITN.

In contrast, few studies have addressed the entomological effects of ITNs on sandflies. To my knowledge four reported studies have tested the impact of ITNs on leishmaniasis under field conditions (Table 4.2), and two additional studies have focused exclusively on entomological impacts (Elnaiem *et al.*, 1999b; Alexander *et al.*, 1995c). Three of the epidemiological trials also carried out entomological evaluations of deltamethrin (25 mg/m²) treated bednets by comparisons of indoor sandfly abundance using sticky traps (Tayeh *et al.*, 1997; Nadim *et al.*, 1995; Alten *et al.*, 2003), but all failed to find any statistical difference, possibly because of small sub-samples (10 houses per treatment) (Tayeh *et al.*, 1997; Nadim *et al.*, 1995) or because sandfly abundance pre-intervention was different in treatments and controls (Alten *et al.*, 2003). This last study also reported no impact on outdoor sandfly abundance. Nevertheless, all four trials noted a reduction in CL incidence with ITNs. In Syria (Tayeh *et al.*, 1997) the incidence in the third year post-intervention was 6.1% (118 / 1929) in the control village compared with 1.2% (21 / 1769) in the villages with ITNs. In Iran (Nadim *et al.*, 1995), although the

Table 4.2 Summary of field trials on insecticide treated bednets for sandflies and cutaneous leishmaniasis control.

Country and locality (Reference)	Vector species	Insecticide (Dose)	Study design		Outcomes (Sampled technique)	Evaluation frequency (Duration)	Results
			Type of treatment	(Sampled unit; sample size and treatments ^a)			
Afghanistan, Kabul (Reyburn et al., 2000)	<i>P. sergenti</i>	Permethrin EC (500 mg/m ² for ITB) (1000 mg/m ² for bed sheets)	Polyester bednets, 154 mesh, bed sheets and indoor house spraying.	Unmatched randomized study. (Household; 200 ITB, 200 bed sheets, 200 HSP, 400 CON).	Epi: CL incidence (interview survey).	Epi: 3 times (15 months).	Epi: Reduction in incidence from 7.2% in the CON to 2.4% in ITB, 2.5% amongst bed sheets and 4.4% in the house spraying. ITB and bed sheets were equally effective, 65% protective efficacy, while HSP gave 40% protective efficacy.
		Lambda-cyhalothrin WP (30 mg/m ² for HSP)		Epi: Study amongst non-immune population. 842 persons for ITB treatment, 730 persons for bed sheets, 813 persons for house spraying and 1281 for CON. Comparison pos-int.			
Syria, Aleppo (Tayeh et al., 1997)	<i>P. sergenti</i> <i>P. papatasi</i>	Deltamethrin SC (20 - 25 mg/m ²)	Polyester bednets, 156 mesh. Reimpregnation once a year.	Cluster randomized study. (Village; 2 ITB, 2 UTB) Ent: Sub-sample of houses (10 ITB, 10 UTB) which were Randomly sampled within a group of houses with high sandfly abundance. Comparison pos-int. No control pre- int.	Ent: Sandfly indoor and outdoors abundance (sticky traps). Epi: CL incidence (questionnaires).	Ent: 12 times for 6 months / year (2 years). Epi: 17 times. Pre-int: once. Post- int: 4 times (2 years); monthly (1 year).	Ent: Not differences in abundance of all sandflies or <i>P. sergenti</i> females. Epi: Lower CL incidence (third year pos-int) in the ITB treatment, 1.2% (21/1769), compared with the pre-int, 5.1% (103/2035), and the CON pos-int 6.1% (118/1929).
				Epi: 252 houses with ITB, 256 with UTB. Comparison pre-int vs. pos-int and pos-int.			

Table 4.2 continued.

Country and locality (Reference)	Vector species	Insecticide (Dose)	Study design (Sampled unit; sample size and treatments ^a)		Outcomes (Sampled technique)	Evaluation frequency (Duration)	Results
			Type of treatment				
Iran, Bam city (Nadim et al., 1995)	<i>P. sergenti</i>	Deltamethrin (25 mg/m ²)	Cotton and nylon bednets, covering all inhabitants in each house.	Cluster randomized study. (Village; 1 ITB, 1 CON, no replicates)	<u>Ent</u> : Sandfly indoor abundance (sticky traps).	<u>Ent</u> : 12 times (4 months).	<u>Ent</u> : Not reduction in indoors sandfly abundance. Failed in the setting place for the traps.
				<u>Ent</u> : Sub-sampled of houses (10 ITB, 10 CON). Comparison pos-int. Not control pre-int Monthly bioassays to test insecticidal power. <u>Epi</u> : 1121 persons for ITB and 1293 for for the CON. Comparison pos-int.	<u>Epi</u> : CL incidence (interview surveys).	<u>Epi</u> : 5 times (1 year).	<u>Epi</u> : Reduction in CL cases pos-int from 0.03% (26/848) in the CON compared with 0.01% (10/728) in the ITB treatment, but weak significance ($p = 0.05$).
Turkey, Anliufara city Alten et al., 2003)	<i>P. segenti</i> <i>P. papatasi</i>	Deltamethrin (25 mg/m ²)	Polyester bednets, 156 mesh. Reimpregnation twice a year.	Cluster non-randomized study. (Village; 2 ITB, 2 CON, 1 UTB) <u>Ent</u> : Sub-sample of houses (20 ITB, 20 CON, 10 UTB) which were randomly sampled. Comparison pos- int. No control pre-int. <u>Epi</u> : Population of 10468 inhabitants included. 2259 householders. Comparison pre-int vs. pos-int.	<u>Ent</u> : Sandfly indoor and outdoor abundance (sticky traps, CDC light traps). <u>Epi</u> : CL incidence (interview surveys).	<u>Ent</u> : 15 times (1.5 years). <u>Epi</u> : 8 times (1 year).	<u>Ent</u> : Not reduction in sandfly indoor or outdoor abundance. <u>Epi</u> : Comparison pre-int vs. pos-int: Significant reduction in CL incidence from 1.9% (44/242) to 0.04% in one treated village and from 2.35 (33/1519) to 1.3% in the other. Comparison pos-int: Significant difference of one of the treated village with its control, but not in the other.

^a Treatments: ITB: Insecticide treated bednet, UTB: Untreated bednet, CON: Control, without intervention; Ent: Entomological; Epi: Epidemiological; HSP: House spraying; Pre-int: Pre-intervention; Pos-int: Post-intervention.

incidence was higher in the control village, 0.03% (26 / 848), compared with the treated village, 0.01% (10 / 728), the results were inconclusive because there was no base line data, there was no replicas and the incidence in the control group was very low. In Turkey (Alten *et al.*, 2003) there was a significant reduction in CL incidence following the introduction of ITNs in two treated villages, from 1.9% to 0.04% and from 2.3% to 1.3% while in the control villages there was no concurrent reduction in incidence. Nevertheless, the reduction in CL incidence was not detected in all treated villages, and the treated and control villages were not well matched by pre-intervention incidence.

The most conclusive ITN trial was the household study in Kabul (Reyburn *et al.*, 2000), where other two treatments (house spraying and impregnated bed sheets) were tested. In this study CL incidence was reduced from 7.2% in the control group to 2.4% in the houses with ITNs. This study did not evaluate entomological variables.

Two other ITN trials in Colombia have been referred to in the literature but have not been fully described. In the Caribbean Coast, deltamethrin treated bednets (26 mg/m²) reportedly reduced human exposure to *L. evansi*, the local vector of VL (Velez *et al.*, 1999). In Tumaco, a matched randomized study in 20 villages (10 treated, 10 control) evaluated the impact of a package of vector control measures (deltamethrin, 26 mg/m², treated bednets, repellents, and whitewash treatment of peridomestic tree trunks) on CL incidence. CL incidence was reduced after one year post-intervention, with a risk ratio of 0.42 (0.14 - 1.26) (Alexander *et al.*, 1995b; Rojas 2001), but it was not possible to assess the impact specifically attributable to the ITNs.

The two exclusively entomological studies of the impact of insecticide treatment of bednets both demonstrated significant protection. A field study in Sudan (in an *Acacia* thicket), which compared the effect of ITNs (154 /inch mesh, apparently) with untreated bednets and with no bednets, showed that lambda-cyhalothrin (10 mg/m²) treated bednets provided complete protection (0 bites) from *P. orientalis*, while the average biting rates with the untreated bednet and without a bednet were 6.9 s/p/n and 32 s/p/n respectively (Elnaiem *et al.*, 1999b). Finally, in a field study in the Valle del Cauca, Colombia, deltamethrin (26 mg/m²) treated bednets (64 /cm² mesh) used indoors reduced significantly the biting rate of sandflies (mainly *L. youngi*) inside (0.14 s/p/h) the bednet compared with the biting rate inside the same room but outside the net (1.9

s/p/n) or in unprotected rooms (3.29 s/p/n) (Alexander *et al.*, 1995c); and 40% of sandflies found resting on ITNs were knocked down by 24 hours.

Further evidence that insecticide treatment of bednet material should enhance the barrier effect of the bednet and/or reduce the bloodfeeding rate of those sandflies that do cross through the netting comes from a series of laboratory and field experiments with insecticide treated curtains. In laboratory bioassays, wild *P. perniciosus* and *P. papatasi* confined in a cage were stimulated, using a bait, to cross a treated curtains (cotton, 0.5 cm mesh size) to test the effect of treatment on repellence, feeding rate and mortality (Micelli *et al.*, 1988; Maroli and Majori 1991). The results showed that permethrin (1000 mg/m²) had a low repellent effect on both sandfly species, but reduced the feeding rates by 67% and 80% (for *P. perniciosus* and *P. papatasi*, respectively) and caused high 24 h mortality (> 90% for each species). Analagous experiments with *L. longipalpis* found that insecticide treatment of wide (0.5 cm) mesh synthetic curtain material prevented 80% of sandflies crossing through to a hamster bait, reduced the feeding rates of those that did by about 90%, and led to 100% mortality in 1 hour (Oliveira *et al.*, 1994).

This barrier effect was also demonstrated by field tests of deltamethrin (26 mg/m²) or lambda-cyhalothrin (12.5 mg/m²) treated curtains on *L. youngi* in Colombia and Venezuela, respectively (Alexander *et al.*, 1995c; Kroeger *et al.*, 2002) and field tests of permethrin (1 g/m²) treated curtains on *P. perfiliewi* in Italy (Maroli and Lane 1987; Maroli and Majori 1991) and on *P. duboscqui* in Burkina Faso ((Majori *et al.*, 1989; Maroli and Majori 1991). However, only high doses of deltamethrin treatment (1 g/m²) were sufficient to cause a significant barrier to *L. ovallesi* or *L. spinicrassa* in Venezuelan field trials (Perruolo 1995; Feliciangeli *et al.*, 1995; Campbell *et al.*, 2001). Similarly, in Sudan permethrin treatment of curtains (from 0.5 – 1.5 g/m²) failed to reduce *P. papatasi* entering rooms, but significantly reduced indoor biting rates, with zero bites recorded in rooms using curtains treated with 1.5 g/m² during the first two months post-impregnation compared to 18.5 bites/2h/person in rooms without curtains and 4.8 bites/2h/p in rooms with untreated curtains (Elnaiem *et al.*, 1999a).

4.1.3 Outline and rationale of studies

The present chapter describes four field studies in Huila. Study 1 directly measured the entomological effects of the ITNs in the field but under controlled conditions (“efficacy”), i.e. with field assistants as bait, and ensuring that bednets are used correctly. Study 2 compared the entomological effect of ITNs and house spraying in the field under “natural” conditions (“effectiveness”), i.e. applying the intervention in the villages, and collecting outcome data indirectly from CDC light traps. Study 3 was designed to validate the method for sandfly sampling in the second study. Study 4 measured the residual lethal effect of the insecticide on ITNs and house spraying, as applied during the second study.

Study 1 (unlike study 2) allowed the direct measurement of the impact of the insecticide treatment on repellency, mortality and diversion. The identification of these effects then helped in the interpretation of the results from study 2, which compares the impact of ITNs and house spraying under natural conditions. Study 1 compared the impact of an ITN with an untreated bednet, and therefore the mesh size of the bednets was larger than that used in study 2, in order to allow sandflies to cross the net and so assess the direct impact of the insecticide treatment on the biting rates inside the bednets. As these bednets were new and were used by research assistants, a fine mesh net would have prevented entry of sandflies irrespective of the insecticide treatment. However under natural conditions bednets get torn or are not well used so leaving gaps. In study 2, the effectiveness of ITNs and house spraying against *L. longiflocosa* was compared under “real” conditions, i.e. as used by the community. Epidemiological evaluation was not possible due to logistic constraints on the cost and length of the study. Study 2 used CDC light traps to monitor the entomological impact, and Study 3 was designed to confirm that CDC light trap captures are correlated with human landing and thus are a valid indirect measure of human landing. Furthermore, it was important to demonstrate whether or not the effectiveness of indoor CDC light traps catches was affected by the presence of ITNs or by house spraying (due to the possible excito-repellent effect of pyrethroids). In short, it was necessary to demonstrate that the relationship between CDC trap catches and human-landing rate is the same in sprayed and unsprayed houses (Davies *et al.*, 1995). Finally, in order to ensure that any possible failure to detect an entomological impact of the ITNs or house spraying was not simply due to loss of

insecticide effectiveness, field bioassays were carried out four months after treatment (in study 4).

4.1.4 Objectives

The studies described in this chapter have the overall aim to evaluate the use of ITNs as an alternative to house spraying for the control of CL vectors in the sub-Andean region of Huila department. The specific objectives are:

- 1) To describe the potential entomological effect on *L. longiflocosa* (i.e. efficacy) of wide mesh (64 /cm^2) lambda-cyhalothrin treated bednets, by comparison of sandfly indoor abundance, human landing rates inside and outside the bednets, and sandfly mortality at 0 h and 24 h after each test.
- 2) To compare the impact (i.e. effectiveness) of ITNs and house spraying, both with lambda-cyhalothrin, on indoor sandfly abundance, percentage of blood-fed females, percentage of fully-fed females and the human blood index as detected by CDC light traps.
- 3) To validate the use of CDC light traps as an indirect measure of human landing rates.
- 4) To determine the residual lethal effect of ITNs and house spraying, both with lambda-cyhalothrin, for up to 4 months post-treatment under field conditions.

4.2 METHODS

4.2.1 Study 1: Potential entomological effect of insecticide treated bednets

4.2.1.1 Study design

The study was carried out in August 2001 in two houses, "A" and "B", in La Troja village (See Chapter 3, section 3.2 for general description). The houses were selected based on their similar high sandfly abundance, 149 s/LT/n and 133 s/LT/n, respectively, described in Chapter 3. The houses were located on the slopes of opposite mountains divided by a narrow canyon at approximately the same altitude (1650 m a.s.l. and 1700

m a.s.l., respectively) and 1350 m apart. House "A" was inhabited by two adults, each one sleeping in one room; while house "B" was inhabited by eight people, two adults and two children sleeping in the main bedroom, and four children in another bedroom.

The study design was a cross-over, where the use of an ITN (set up in the main bedroom of one of the two selected houses) was compared with one untreated bednet (set up in the main bedroom of the other house). The study was carried out during 10 consecutive days, where treatments were allocated alternatively to houses "A" and "B". Two nylon bednets for single beds (1.94 m x 1.49 m x 1.15 m) were used with mesh 64 /cm². Impregnation was with lambda-cyhalothrin, CS, 2.5%, 25 mg/m² (as described in section 4.2.2.3, except that a single bednet was dipped using a plastic bag). Each night, the bednet was set on the floor, over a camp bed with the borders of the net folded under the camp bed. A light-coloured paper sheet (changed nightly) was set under the camp bed extending for 30 cm around (Figure 4.1). To minimize possible cross-contamination, the floor of each bedroom was covered by a 3 x 2 m plastic sheet before setting up the bednets. Secondly, setting up and dismounting of bednets were carried out using rubber gloves. Finally, for each treatment the same set of materials (e.g. camp bed, mouth aspirators, and gloves) were always used.

The comparison of treatments included the impacts on human landing rates, sandfly abundance and mortality inside the bednet, outside the bednet, and overall in the bedroom. In each house, human landing catches were carried out by two volunteers, one inside the bednet (Figure 4.1) and one 1 m outside the bednet (Figure 4.2). Each volunteer exposed their forearms and lower legs and captured the sandflies using a mouth aspirator with a help of a torch. Catches lasted three hours, from 22:00 h to 01:00 h. This period of time was chosen after preliminary human landing catches were made in the two houses from 19:00 - 01:00 h (after which catches were not feasible as householders did not want to be disturbed as they slept). The selected 3 hour period was based on: 1) apparent peak hour of sandfly biting rate between 11:00 h to 12:00 h; 2) absence of sandflies before 21:00 h; and 3) suggestion of a decrease in sandfly abundance after 01:00 h (Figure 4.3).



Figure 4.1 Indoor human landing inside a bednet during the study on the efficacy of ITNs. Photo by Raul Pardo.

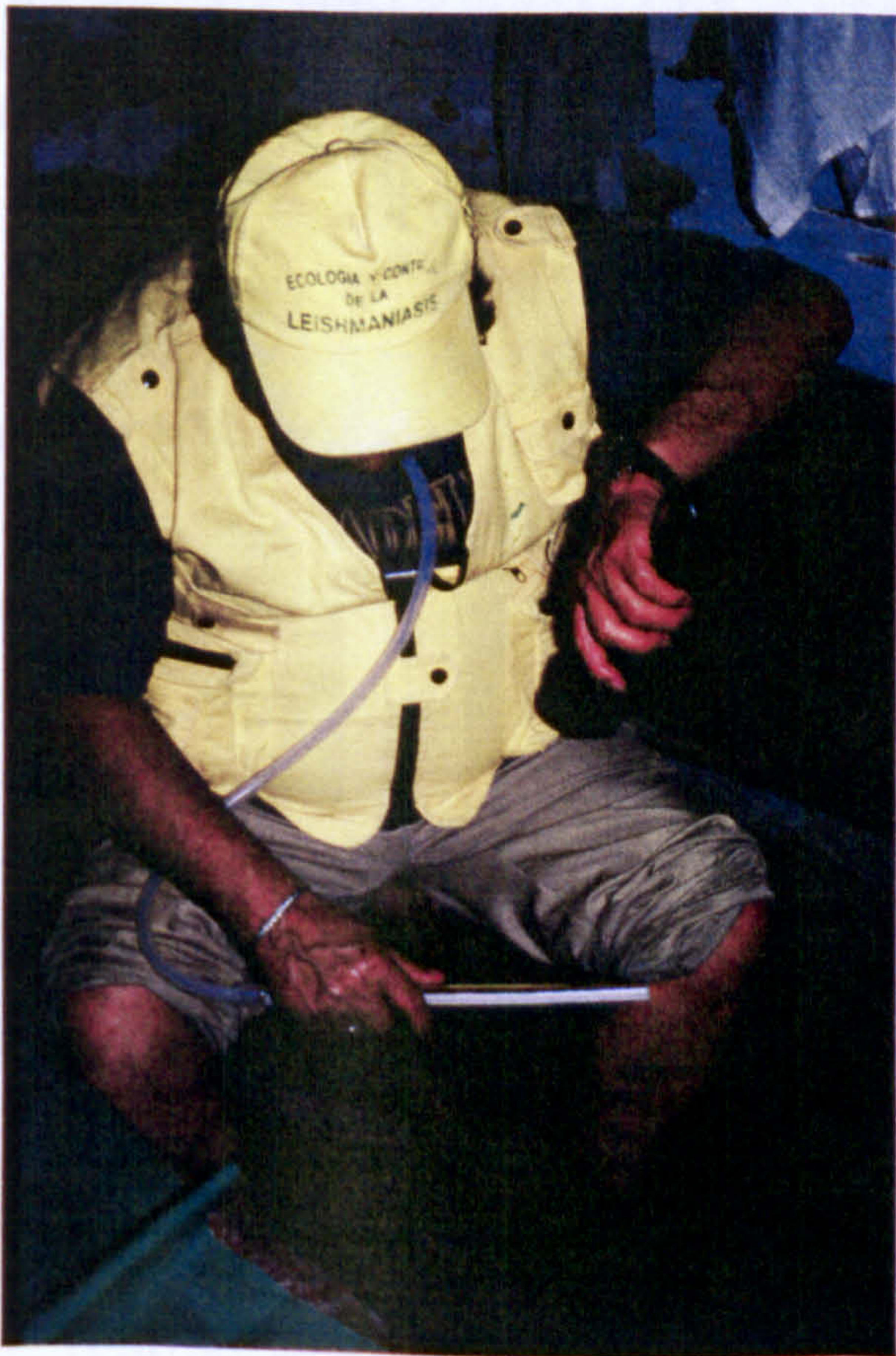


Figure 4.2 Indoor human landing outside a bednet during the study on the efficacy of ITNs. Photo by Raul Pardo.

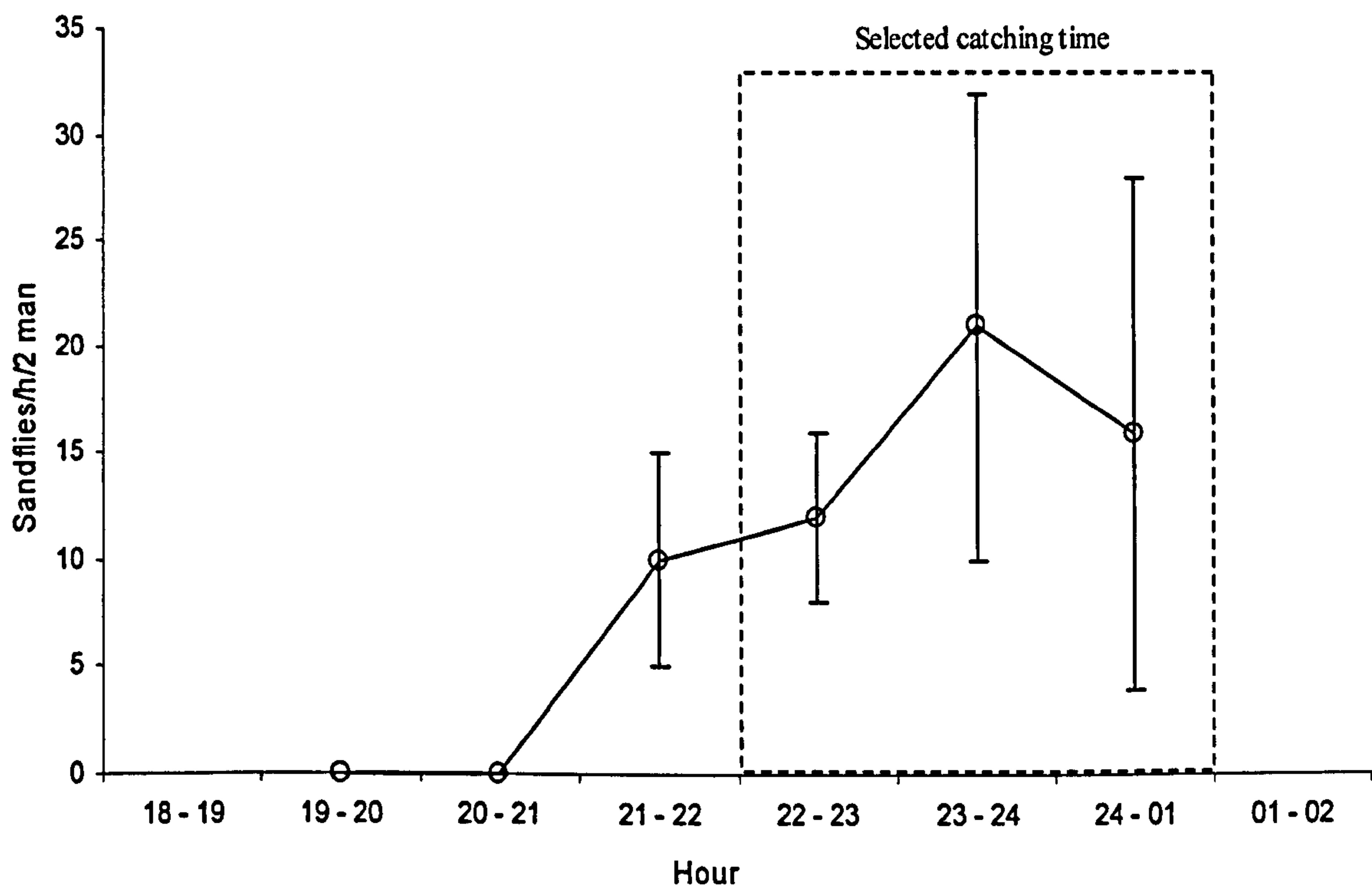


Figure 4.3 Preliminary observations on sandfly biting activity indoors in La Troja village (Baraya municipality), providing a rationale for the catching time (dotted rectangle) selected for the ITN efficacy study. Data correspond to one night of simultaneous human landing catches (19:00 - 01:00 h) by two volunteers in each of the two study houses. Bars indicate ranges.

Each night at 01:00 h, after the human landing catches had finished, sandflies resting in the inner and outer surfaces of the bednets were collected, knockdown or dead sandflies lying on the floor in a 30 cm radius surrounding the bednet were collected, and finally sandflies resting on the walls of the bedroom were collected (for 10 min). Householders were encouraged to maintain their normal sleeping habits during the study period, but some changes were inevitable. In house "A", both people slept in the bedroom not being used by the entomologists; while in house "B", three people (one adult and two children) slept under untreated bednets in the main bedroom (as they normally did) beside the two volunteers.

All sandflies were stored separately prior to identification. Living sandflies were kept for 24 h (when death rates were recorded) in containers with wet plaster at the bottom, and provided with water and saturated sucrose. The containers were kept in polystyrene

boxes. Identification of specimens was carried out as described in Chapter 3 (section 3.3.2).

4.2.1.2 Statistical analysis

A total of six variables were evaluated. In general, these refer to the complete sandfly collection each night, but – where stated – these variables are measured for sub-samples: namely, those collected inside the net (whether human landing, resting or knocked down) or those collected outside the net (whether human landing, resting or knocked down). The six variables are:

- 1) Landing rate: the number of *L. longiflocosa* females caught by human landing (/person/3h).
- 2) Percentage of landing females: the number of *L. longiflocosa* females (x 100) captured by human landing divided by the total number of females of this species.
- 3) Immediate mortality: the number of knocked sandflies (dead and alive) found lying on the floor at 01:00 h, divided by the total number of sandflies.
- 4) 24 h mortality: the total number of dead sandflies (immediate plus 24 h) divided by the total number of sandflies.
- 5) Percentage of females resting on the walls: The number of *L. longiflocosa* females resting on the walls divided by the total number of females.
- 6) Total abundance: the total number of sandflies collected.

Variables dealing with abundance or landing rates are presented as Williams' geometric means (GM) with units of females or sandflies per person /3h (f/p/3h, s/p/3h), unless stated otherwise. Percentages, including mortality, are presented. The analyses were carried out by multivariate statistical analysis using GLIM in Stata 7 software (Stata Corporation 2001). For counts, most of the analyses were carried out on the log transformed data, $\ln[x + 1]$, with the assumption of Normal errors. The only exception was the analysis of *L. longiflocosa* females landing inside the net, which used the untransformed data with the assumption of Poisson errors. Proportions were analyzed with the assumption of binomial errors. All analyses were adjusted by house. Chi-squared tests (χ^2) with the Yates' continuity correction were used to analyze some proportions where a low number of sandflies was involved.

4.2.2 Study 2: Comparison of insecticide treated bednets and house spraying for sandfly control

4.2.2.1 Study design

The trial was conducted between January and July 2001 in the same villages as the risk factors study (see Chapter 3): La Troja (Baraya municipality) and El Cedral (Neiva municipality). Brasilia village was excluded for security reasons. The trial was at the household level, applying a random matched design. The main outcome measurement was sandfly indoor abundance recorded by CDC light traps (as an indirect measure of human biting). Secondary outcome measurements were the proportions of blood-fed females, of fully-fed females and of females with human blood (human blood index, HBI). Three treatments (ITNs, spraying, and controls) were evaluated simultaneously, with treatment selection randomized amongst “triplets” matched by village and pre-intervention sandfly abundance (as measured in Chapter 3). Amongst the 265 houses sampled in Chapter 3, for each selected village, the houses were sorted by decreasing order of abundance and grouped in triplets. Control households all received free ITNs after the final post-intervention sampling. The inclusion of the control group is considered ethical since it represents "no change to current practices", and diagnoses and treatment were available during the study period. Also, the efficacy and effectiveness of the antivectorial measures are being investigated in this study and have not yet been established against *L. longiflocosa*.

The number of triplets to include in the trial had been initially decided on the basis of a sample size calculation based on data from 45 houses sampled in the study area during the study described in Chapter 2 (Table 4.3). The primary outcome was the impact of treatments on the log transformed abundance of *L. longiflocosa* amongst the 22% of houses with highest abundance (in Chapter 2); the mean and variance were 3.89 [ln sandflies]/house and 0.504, respectively. Sample size was calculated based on the formula:

$$n = [(z_1 + z_2)^2 (\sigma_1^2 + \sigma_2^2)] / [\mu_1 - \mu_2]^2$$

In this case for 90% power and significance at $p < 0.05$ this simplifies to: $10.5 [\sigma_1^2 + \sigma_2^2] / [\mu_1 - \mu_2]^2$. If there is at least a 50% reduction in the geometric mean (and assuming that the variance will drop around 25% as a result of this), using the before

Table 4.3 Females of *Lutzomyia longiflocosa* collected with CDC light traps (LT) inside 45 rural houses in the municipalities of Baraya, Neiva and Algeciras (as described in Chapter 2).

Number of females (LT/ night)	Observed frequency	Cummulative percentage
160	1	2
97	1	4
89	1	7
69	1	9
45	1	11
41	1	13
34	1	16
30	1	18
20	1	20
16	2	24
14	1	27
9	1	29
8	1	31
7	1	33
6	2	38
5	4	47
4	4	56
3	2	60
2	3	67
1	4	76
0	11	100
Total	45	

formula, a sample size requirement of 20 houses in each treatment is estimated (Smith and Morrow 1996), i.e. a total of 60 houses. To identify 60 houses with the mean and variance of the top 22% of houses in the region, it was therefore required to sample a total of 273 (60 / 0.22) houses.

The secondary outcome of the trial was the proportion of sandflies with blood meals. The sample size to detect a 50% reduction in the proportion of blood-fed sandflies was calculated on the basis that 21% of sandflies collected will be blood-fed (as found in the 45 houses from this region sampled in Chapter 2). Sample size was calculated based on the formula:

$$n = [(z_1 + z_2)^2 \cdot 2 \cdot \bar{p} (1 - \bar{p})] / [p_1 - p_2]^2$$
, where $\bar{p} = [p_1 + p_2] / 2$

For 90% power to detect a significant difference (at $p < 0.05$), this simplifies to: $n = [21 \bar{p} \{1 - \bar{p}\}] / [p_1 - p_2]^2$. Using the before formula a sample size requirement of 253 sandflies per treatment was estimated (Smith and Morrow 1996). Based on the 22% “top” houses in the preliminary survey, where the arithmetic mean number of sandflies was 60/house/night, we expected 1200 sandflies in the control group (20 houses sampled once (60 x 20)), of which 252 would be blood-fed. Hence, if the treatments failed to cause any significant reduction in sandfly abundance, the sample size should have been sufficient to identify a 50% reduction in blood-fed proportion.

The third outcome measure was the human blood index. To estimate the number of blood-fed females required to detect a reduction in the proportion of females with human blood it is necessary to know the base line HBI of *L. longiflocosa* females. This is unknown, but we estimated, conservatively, that 50% of the blood-fed sandflies would contain human blood. Using the same formula above, if a 50% reduction in the HBI is caused, there will be 90% power to detect a significant change with a sample size of 79 females ($p < 0.05$). Hence, if the treatments failed to cause any significant reduction in either sandfly abundance or the proportion of blood-fed, a sample size of 252 blood-fed females would be sufficient to detect a 50% reduction in the HBI. For the fourth (minor) outcome measure, the proportion of fully blood-fed females, there is also no base line information. So a similar estimation of sample size to the above explained for HBI was made.

So, 27 triplets with similar abundance within triplets were formed. The first 20 triplets (60 houses, threshold of 21 female sandflies) were considered to be included in the study; the remaining seven triplets were left as spare in case some of the main triplets were lost for any reason. The three treatments (ITNs, spraying and controls) were assigned randomly within each triplet. These treatments were applied in May 2001. Finally, in July 2001 (three months after the treatments were applied), a post-intervention sampling was carried out. During this sampling CDC light traps were allocated to the same bedroom used in the pre-intervention sampling. Unfortunately, because of security reasons in one of the sampled municipalities, only 16 triplets of houses could be sampled. Both sampling occasions were planned in such manner that they coincided, to a large extent, with the two dry seasons when the sandflies seem to

present the highest abundance within the study area (January to February and July to September). Sandfly abundance in other seasons apparently decreases drastically, which could undermine the study.

Finally, it should be mentioned that the practice of control measures by the householders was not an exclusion factor in house selection for the present study. The reasons for this were: (1) most of householders, 82%, according to the pre-intervention sampling (see Chapter 5, Section 5.3.2) used some kind of sandfly control; (2) no long term effect was detected on sandfly abundance or disease as a result of the use of any of the control measures by the householders (Chapter 3, Section 3.5.4.7); (3) in the particular case of bednets, the percentage of householders included in the intervention study which use bednets were relatively low and similar between treatment groups: 19% (3 / 16) for both, house spraying and ITNs, and 25% (4 / 16) for the controls. The possible impact of the few ITNs (deltamethrin, 25 mg/m² owned by the householders in El Cedral village (provided, as mentioned before, by the NHS) was expected to be low because the insecticidal effect was unlikely to have persisted for the two years since impregnation. Furthermore, householders in the house spraying and control treatments apparently did not use their ITNs during the whole intervention study. This could be explained by the seasonal use (time of high sandfly abundance) of the control measures within the study area (Chapter 5, Section 5.3.7). Finally, the households in the ITN cohort only used the treated bednets provided by the intervention trial team during the whole study, keeping out of service the older treated bednets.

4.2.2.2 Insecticide and dose

The insecticide used in the study was the pyrethroid lambda-cyhalothrin (Demand CS, 2.5%, Zeneca U.K.). Pyrethroids are the only insecticides which have been approved by the WHO to be used for ITNs because their high insecticidal potency at low doses, their rapid knockdown effect, and because they are relatively safe for human contact (Zaim *et al.*, 2000). WHO has classified lambda-cyhalothrin (a.i.) in Class II "moderately hazardous". According to the manufacturer the CS (microencapsulated suspension) formula has the advantage of relatively low toxicity, which reduces irritability of mucous membranes and skin in users, as well as contact with the environment, and has a higher residual effect, at least for three months (BASF 2000).

The target dose of 25 mg/m² of insecticide used in the present study was selected based on the experience of a previous study with *L. verrucarum* in Peru where field bioassays, using the WHO contact bioassays cones, on adobe walls showed that indoors spraying with 25 mg/m² of lambda-cyhalothrin caused 100% mortality of wild *L. verrucarum* up to six months (Davies *et al.*, 2000a). In the same study, bioassays testing three lower concentrations (5, 10 and 20 mg/m²) of lambda-cyhalothrin detected a dose response with a LD₉₅ of ca. 20.1 mg/m² for colonized *L. verrucarum*.

4.2.2.3 Application of treatments

a) Bednets

White polyester bednets were used with ca. 0.7 mm mesh size (mesh 163 /cm²). Two types of bednets were delivered: single bednets (11.0 m² area [2.02 m x 1.50 m x 1.00 m]) and double bednets (13.5 m² area [2.02 m x 1.50 m x 1.50 m]). Impregnation was carried out by batches (17 to 33 bednets of the same size per batch) by the research team using lambda-cyhalothrin CS, with the target dose of 25 mg/m², following the procedure described by Lines (1996). Impregnation was carried out in an open, well ventilated area. The personnel participating in the impregnation wore long rubber gloves, rubber aprons and boots, and protective glasses. All personnel washed their hands and clothing after the dipping was finished.

In addition to the area of material, the water absorbed by each bednet must be known to calculate the target dose of insecticide. The volume of water absorbed by each of 5 bednets was calculated by: (a) soaking the bednet in 4000 ml of water in a bucket; (b) wringing out the bednet, and allowing to drip so the excess of water fell back into the bucket; (c) measuring the remaining volume of water in the bucket; and (d) measuring the difference. A single bednet absorbed a mean of 466 ml and a double bednet a mean of 549 ml. These figures were then used to calculate the total amount of water required to dip each batch (minus the volume of insecticide to be added). To calculate the amount of insecticide required per batch, (i) the mean area of the bednet was multiplied by the target dosage in mg/m² (i.e. 11.0 x 25 = 275 mg, or 13.5 x 25 = 338 mg for single and double bednets, respectively); (ii) this figure was divided by the concentration of insecticide in mg/ml (as there were 25 g of insecticide per liter of

solution, then the equivalent in mg/ml was 25 and so the required volumes of insecticide were: $275 / 25 = 11$ ml and $338 / 25 = 13.5$ ml , for single and double bednets, respectively); and (iii) these per capita estimates were then multiplied by the total number of each bednet type to be dipped to provide the total amount of insecticide concentrate needed to be mixed into the water. For each single bednet 11 ml of insecticide concentrate were mixed with 455 ml of water (total = 466 ml) and for each double bednet 13.5 ml of insecticide were mixed with 535.5 ml of water (total = 549 ml).

Each bednet was soaked completely for a few seconds, wrung thoroughly so the excess of fluid dripped into the dipping container; and then folded and hung for a few seconds to allow a final drip. Bednets were then folded once and laid on plastic sheeting. The bednets were turned occasionally (at ca. 40 min intervals), and when almost dry they were hang up to speed up the process. When the bednets were completely dry, they were packed in sealed plastic bags until they were delivered. Bednets were allocated according to the number of beds used by each household (Figure 4.4) and householders were instructed about the appropriate use of the bednets. They were also requested not to wash the bednets during the study period.

Before the bednets were delivered, bioassays were carried out in order to test the effectiveness of the insecticide, using transparent plastic cones (WHO 1975). Seven groups of approximately ten females of *L. longipalpis* were exposed to an ITN for 2.5 min . The 24 h mortality was 100% against 2.9% (2 / 71) in a control group.

b) House spraying

Lambda-cyhalothrin CS, 25 mg/m², was applied on walls of bedrooms and ceilings (when present), using a Hudson pump by a trained technician (Figure 4.5). Similar safety measures to those described for impregnation of bednets were taken, with the addition of an overall and balaclava. One litre of solution containing 25 ml of insecticide was enough to cover ca. 25 m². Coverage rates were checked by calculating the ratio of insecticide used to the wall and ceiling area in each house.

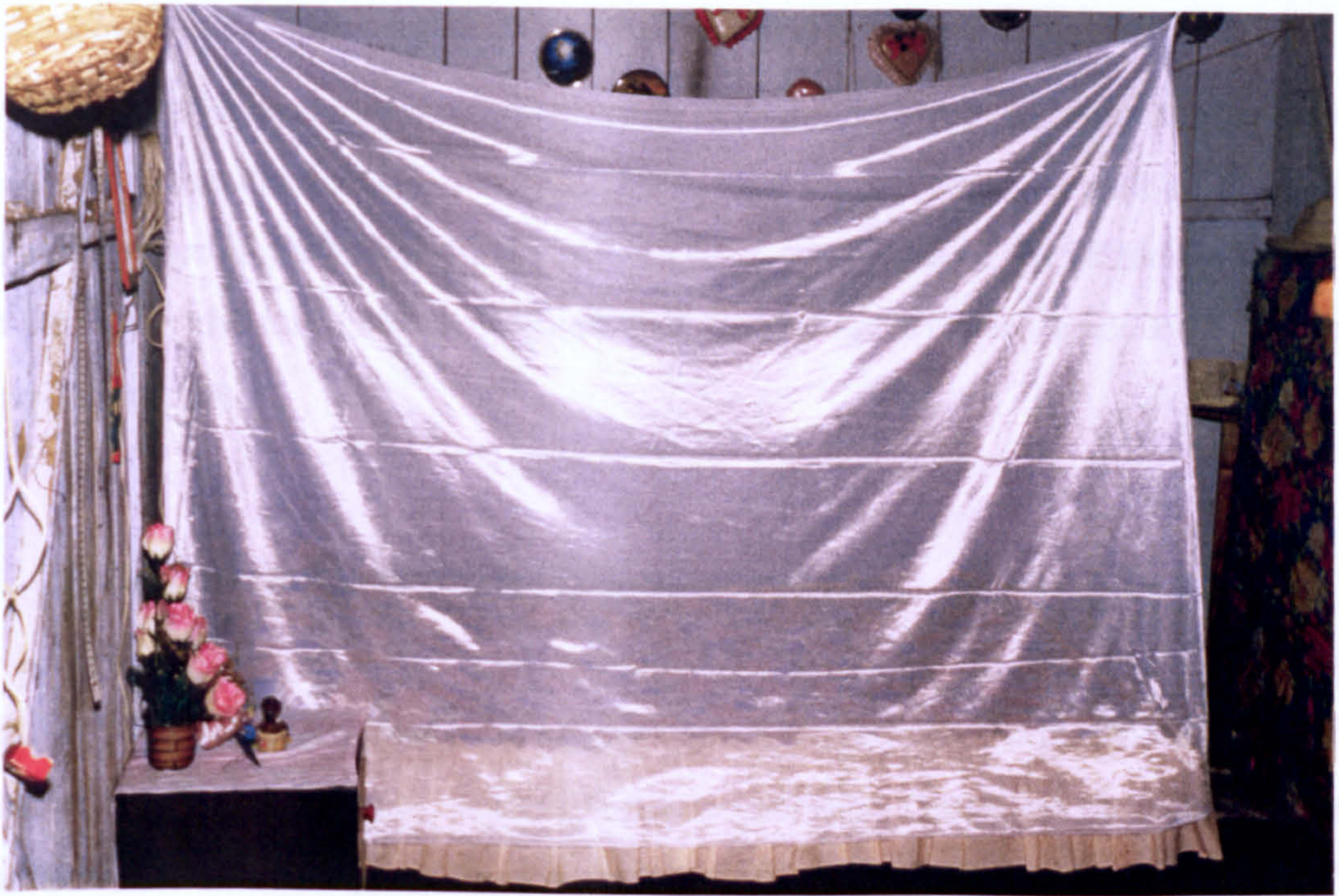


Figure 4.4 Insecticide treated bednet (lambda-cyhalothrin, CS, 25mg/m²) in El Cedral village (Neiva municipality) during the effectiveness study comparing ITNs and house spraying. Photo by Raul Pardo.



Figure 4.5 Indoor spraying (lambda-cyhalothrin, CS, 25 mg/m²) in La Troja village (Baraya municipality) during the effectiveness study comparing ITNs and house spraying. Photo by Raul Pardo.

4.2.2.4 *Householder attitudes to the control measures*

During the post-intervention sampling visits, householders with ITNs or house spraying were interviewed on (1) any benefit or harm they perceived as a result of the control measure applied in their houses, (2) any effect on the health of the residents during the first days after the measure was applied, and (3) any inconvenience caused by sleeping under the bednets.

4.2.2.5 *Processing and identification of sandfly samples*

The sandflies were collected and identified as described in Chapter 3. Blood-fed sandflies were treated separately. During the pre-intervention sampling, fed-females (as explained in Chapter 3) were scored by blood condition (fresh, bright red or digested, dark brown or black) and amount (percentage of abdomen with blood: low (< 25%); half (25 - 75%); or full (> 75%). Some of the blood-fed abdomens were squashed on filter paper, and the remainder were stored in 70% ethanol as numbers were unexpectedly high (23% of all females collected had bloodmeals). During the post-intervention sampling, blood-fed flies were scored as before, and stored in plastic boxes with silica gel under cool conditions until processed in the laboratory.

Human blood identification was carried out by the precipitin ring test (Weitz 1960) on females with half of full bloodmeals. The precipitin ring test is based on the reaction between a layer of antigen (extract of blood sampled) and another of antiserum (from the species of interest). A positive reaction is indicated by an insoluble precipitate (result of the insoluble product formed when antigen and antibody are united) which is visible as a white band or ring. In brief, the procedure applied to the stored blood-fed flies was as follows. Sandflies were hydrated in a humid chamber for 1 - 2 hours. The last four abdominal segments were removed under a stereo-microscope, using sharp disposable sticks, left overnight in a 10% KOH solution, placed in saturated liquid phenol (C_6H_5OH) in a concave microslide for at least 10 min, and then identified based on genital morphology using the usual keys (Young 1979; Young and Duncan 1994).

After, the remaining part of the abdomen (containing the bloodmeals) was cut off and transferred to a phosphate buffer solution (PBS), pH 7.4 (P- 4417, Sigma) where it was macerated with a grinder (K-749521-1500, Anaquen). The amount of PBS solution for

each abdomen varied according to the amount of blood in order to reduce the variation in blood quantity among samples in the final mix. So half blood abdomens were diluted in 100 µl PBS and full abdomens in 200 µl. Bloodmeals smeared onto filter paper were similarly soaked in PBS solution, and all samples were left overnight at 6°C, after which the extracts were centrifuged at 5000 rpm/5 min.

One hundred µl of human blood antiserum (H8765, Sigma), in dilution 1:12, was added to a glass tube (50 mm x 5 mm diameter), over which 100 µl of the extract being tested were layered (carefully minimizing the possibility of mixing of the reagents). The result was read at 30 min and 60 min. Samples were considered positive if the band or ring was observed in at least one of the two readings (Figure 4.6). The test was carried out on batches of 15 to 30 samples at a time. For each batch three controls were included (all *L. longipalpis*): one positive control (human-fed female), and two negative controls (hamster-fed, and unfed-females). Controls always gave the expected results.

Preliminary tests were carried out to determine the optimum antiserum dilution by measuring both sensitivity and specificity for human blood detection using *L. longipalpis* as the gold standard. *L. longipalpis* females (from the sandfly colony of Liverpool School of Hygiene and Tropical Medicine) fed on human or hamster, killed 12 h after feeding, and processed in the same way as the sandflies collected in the field, were used in the tests. The following dilutions of human antiserum were tested: 1:2, 1:4, 1:8, 1:10, 1:12 and 1:14. The two extreme dilutions were tested against horse and dog antigens (from filter paper smears of blood and serum, respectively). Based on these tests the dilution 1:12 was selected for the field samples. This dilution did not present any cross reaction with the tested antisera.

4.2.2.6 Statistical analyses

The effect of treatment on six variables was evaluated:

(1) Mean number of sandflies (s/LT/n, or f/LT/n); (2) mean number of fed females (f/LT/n); (3) Mean number of fully-fed females (f/LT/n); (4) percentage of fed females (i.e. fed/total); (5) percentage of fully-fed females (i.e. fully feds/all feds); (6) proportion of blood-fed females with human blood (i.e. human bloodmeals/all bloodmeals tested).

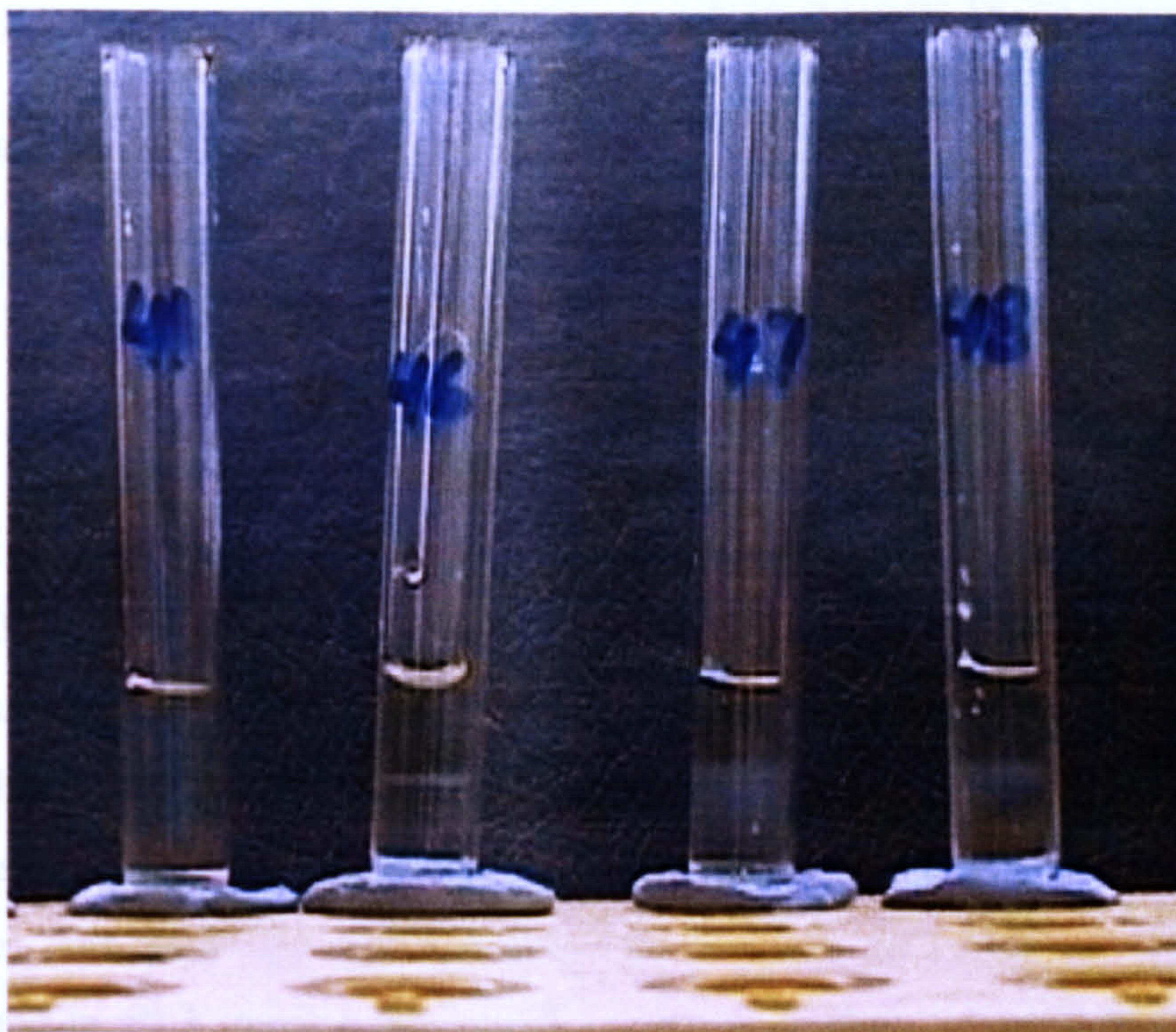


Figure 4.6 Precipitin ring test of blood-fed sandfly females. From left to right: First tube is a negative control, the others are positive samples for human blood. Photo by Raul Pardo.

As previously, the analyses focused on the dominant species *L. longiflocosa* and (where data were sufficient) *L. nuneztovari*, and were carried out by multivariate statistical analysis using GLIM within the Stata 7 software (Stata Corporation 2001). Validation of all models was carried out, as usual, by checking the residuals plots and Quantil-Quantil plots. Post-intervention abundance data (total females or sandflies, fed-females, and fully-fed females) were analyzed using the log transformed values, $\ln[x + 1]$, with the assumption of Normal errors, for *L. longiflocosa*, and on the raw data with the assumption of Negative binomial errors for *L. nuneztovari* (see Chapter 3) adjusting for pre-intervention data (where necessary). Proportions (fed, fully-fed, or human fed) were analyzed by logistic regression with the assumption of Binomial errors, adjusting by triplet. Fed females collected during the pre-intervention sampling not recorded to species, and all were assumed to be *L. longiflocosa* (due to its overwhelming dominance). The few females unidentified due to damage were also assumed to be *L. longiflocosa*. Comparisons of human blood indices controlled for blood condition and amount. Pre-intervention HBIs for the three treatment groups were compared by χ^2 and the Fisher's exact tests, as sampled sizes were small.

4.2.3 Validation of use of CDC light traps as substitutes for human landing

4.2.3.1 Study design

This study was carried out in conjunction with the post-intervention sampling in July 2001. Forty eight houses in La Troja and El Cedral villages (16 matched triplets: three treatments per triplet: ITNs, house spraying and control houses) were divided in 8 groups of 2 triplets (6 houses) each. Each group was sampled for two consecutive nights. On the first night, in one triplet sandflies were sampled with CDC light traps for 13 h (18:00 - 7:00 h). In the other triplet, on the same night, indoor human landing catches (outside the bednet, for the houses with this treatment) were carried out by one volunteer/house, who exposed forearms and lower legs and captured the sandflies using a mouth aspirator, from 19:00 - 21:30 h (sampling beyond this time being logistically impossible). The following night the sampling methods were switched. This procedure was repeated in all 8 groups. All sandflies were identified by the usual procedures as described in Chapter 3.

4.2.3.2 Statistical analysis

The analysis compared the log transformed abundance of *L. longiflocosa* females caught by CDC light traps ($\ln\{f + 1\}/LT/n$) and the log transformed human landing rate of the same sandfly species ($\ln\{f + 1\}/p/2.5\text{ h}$). The analysis focused on *L. longiflocosa* females as the other species were very rare, and only females were caught landing on humans. Males were not analyzed because they were absent from the human landing. Initially the association between the two sampling methods was evaluated using the Pearson's correlation coefficient (r). Effect of treatment on the efficacy of the two sampling methods was analyzed by comparison of the log transformed ratios, $\ln\{LTC + 1\} / \{HLC + 1\}$, using generalized linear models (GLIM) with the assumption of Normal errors, adjusting by triplet. To test if the relative sampling efficiency of the CDC light traps was affected by *L. longiflocosa* female density, the log transformed ratio was plotted against a joint estimate of sandfly abundance, $[\ln\{LTC + 1\} + \ln\{HLC + 1\}] / 2$, and the trend tested by r (Altman and Bland 1983). Finally, the direct effect of treatment on the log transformed human landing catches was tested, after adjusting for triplets and assuming Normal errors.

4.2.4 Residual effect of the insecticide

The bioassays took place at 4 months post-intervention in La Troja village (Baraya municipality) from 6 to 8 September 2001. Sandflies used in the bioassays were wild sandflies caught by human landing (all ages, mostly unfed females) in forest. After collection, sandflies were transported to the field station where they were provided with water and sucrose, kept for at least 12 h in order to check their viability and used in the bioassays within 24 h post-collection.

Evaluation of the residual effect of lambda-cyhalothrin was carried out using the WHO contact bioassays cones (WHO 1975), modified by a layer of foam at the cone edges, which were set against the insecticide treated surfaces (bednet or wall) for a specific period of time, taken special care that the sandflies were in contact with the treated surface most of the time by tapping the cone with a stick when a sandfly tried to land on the cone walls.

Assays were carried out on bednets (polyester, 0.7 mm mesh size) located in three houses: (i) 0 months after treatment, i.e. a freshly treated new bednet installed in a house belonging to the control group; (ii) 4 months post-treatment, i.e. in use throughout the intervention trial; and (iii) control, i.e. an un-treated bednet fitted in another control house. All houses were close to the field station so as to reduce sandfly mortality during the transport from the tested houses to the field station, where monitoring of sandfly mortality was carried out. Bioassays were carried out by batches of four to six cones, each containing 11 to 18 wild sandflies, exposed for 3 min on the largest lateral side of the nets (Figure 4.7). To ensure the nets were stretched to allow a better fixing of the cone, a "embroidery ring" was set around each cone.

Bioassays were carried out on walls made of plastered and painted "bahareque" (wall made of a mix of mud and cow manure with an internal framework of bamboo), i.e. the most common type in the study area (see Chapter 3). Assays were carried out in three houses (i) 0 months after treatment, i.e. a freshly sprayed house, that had previously been a control house; (ii) 4 months post-treatment, i.e. a house sprayed at the start of the intervention trial; and (iii) control, i.e. an unsprayed control house. Bioassays were carried out by batches of two to six cones, each containing 12 to 21 sandflies, exposed

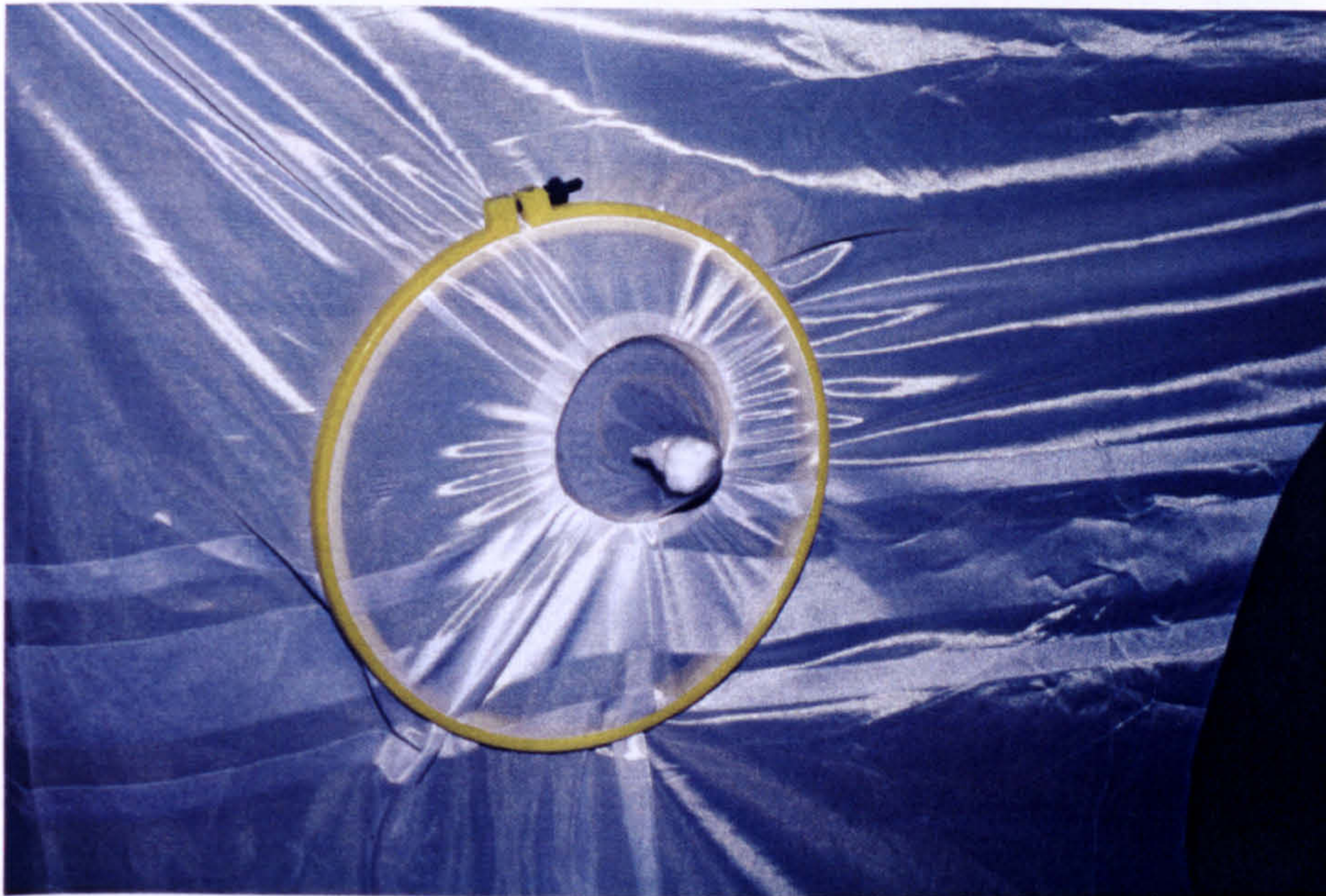


Figure 4.7 Bioassay to evaluate the residual effect of lambda-cyhalothrin CS, 25 mg/m², applied on polyester bednets (0.7 mm mesh size) against *Lutzomyia longiflocosa*. Photo by Raul Pardo.

for 1 h on the walls (Figure 4.8) of one bedroom and one living room per house at approximately 1.5 m height from the floor.

Assays on either nets or walls were mainly carried out between 05:00 h to 09:00 h. After exposure, the sandflies were transferred to plastic containers which had a plaster of Paris layer in the bottom to keep high humidity (Ferro *et al.*, 1998b). The containers were kept in a polystyrene box for 24 h with water and sucrose solution that was made available to the flies. Mortality was recorded at times: 0 h, 1 h and 24 h after exposure. Knocked down insects were taken as dead because preliminary observations showed that they never recovered. All surviving sandflies after 24 h were killed with chloroform. Finally, specimens were counted by sex and preserved in ethanol 70% until they were identified to species (see Chapter 3, section 3.3.2). Statistical analysis was carried out on the total frequencies of dead and alive flies for each treatment, using the χ^2 test with the Yates' correction or the Fisher exact test.

4.3 RESULTS

4.3.1 Potential entomological effect of insecticide treated bednets

A total of 434 sandflies were captured, mostly *L. longiflocosa*, 96.3% (n = 418), with

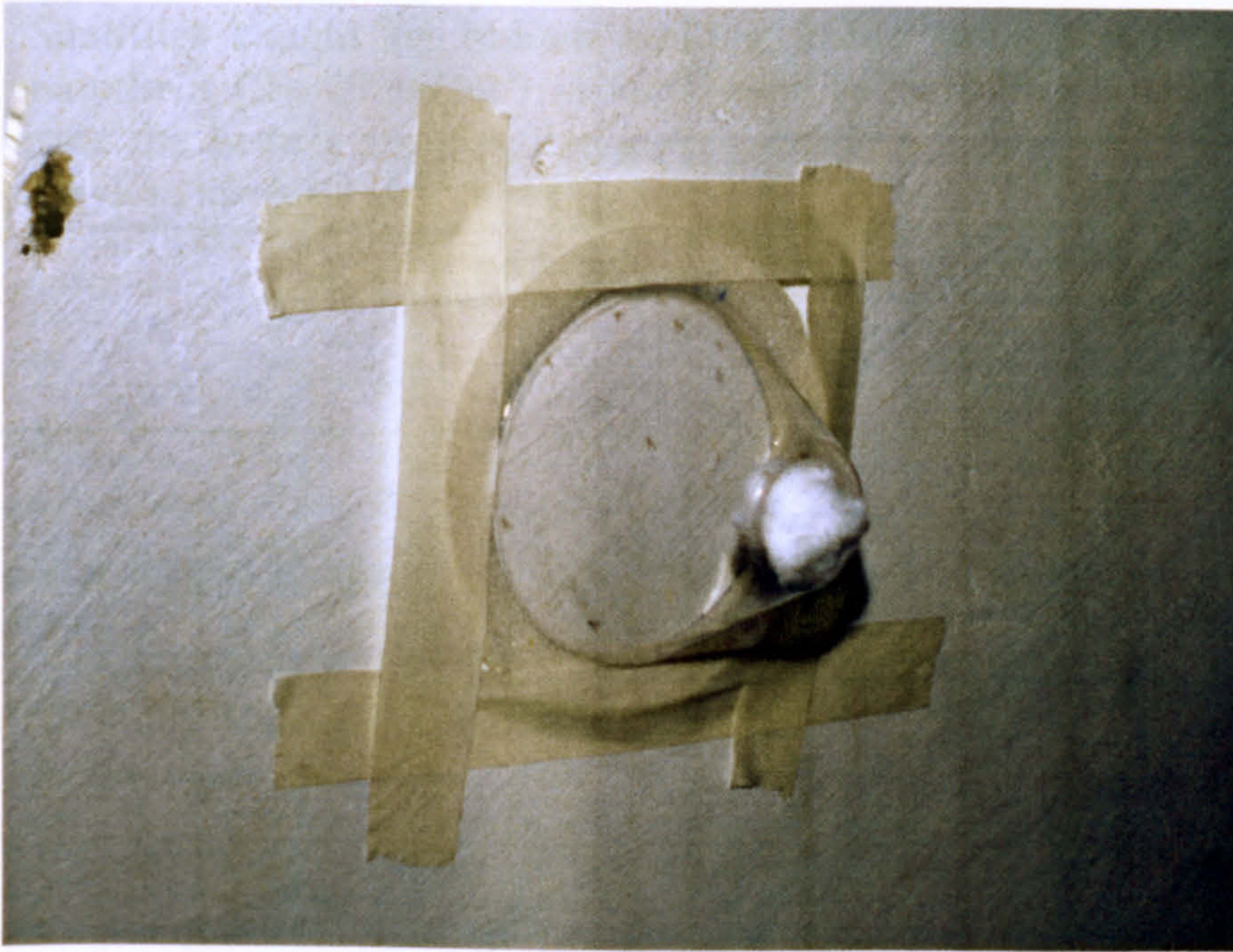


Figure 4.8 Bioassay to evaluate the residual effect of lambda-cyhalothrin CS, 25 mg/m², applied on "bahareque" (mud and straw) plastered and painted walls, against *Lutzomyia longiflocosa*. Photo by Raul Pardo.

few *L. nuneztovari*, 2.5% (11), and *L. columbiana*, 0.7% (3). Two sandflies could not be identified because of damage, and were assumed to be *L. longiflocosa*. Only 9 male sandflies were captured, all identified as *L. longiflocosa*. Hence, the majority of analyses (except mortality, where all sandflies were considered) were carried out on *L. longiflocosa* females. The results are summarized in Table 4.4, which shows that the average number of sandflies landing and resting in the bedroom with the ITN was considerably lower than that in the bedroom where the untreated bednet was used. A knockdown effect is also clear in the bedroom with the ITN. A detailed analysis follows.

4.3.1.1 Female "abundance"

The total number of *L. longiflocosa* females caught in the bedroom with the ITN was significantly lower, 7.9 (4.1 - 15) f/p/3h, compared with the abundance in the bedroom with the untreated bednet, 18.8 (9.0 - 39.6 f/p/3h): $z = -2.14$, $p = 0.033$.

Table 4.4 Sandflies caught by human landing inside bedrooms with an ITN (lambda-cyhalothrin CS, 25 mg/m²) or untreated bednet. One person collected sandflies inside the bednet and another person outside, between 21:30 - 00:30 h (net made of nylon, mesh 64 /cm², n = 10).

Activity or condition	Site	Untreated bednet			Insecticide treated bednet		
		No. sandflies	GM	(95% C.I.)	No. sandflies	GM	(95% C.I.)
Landing	Inside the bednet	16	1.0	(0.18 - 2.5)	1	0.07	-
	Outside the bednet	236	14 ^a	(7.1 - 30)	34	3.0 ^a	(2.0 - 4.4)
Resting	Inside the bednet	10	0.53	(0.08 - 1.6)	1	0.07	-
	Outside the bednet	3	0.20	-	1	0.07	-
	On walls	24	1.3	(0.18 - 3.3)	3	0.20	-
Knockdown	Inside the bednet	0	-	-	25	1.4	(0.24 - 3.6)
	Outside the bednet	17	0.34	(-0.31 - 1.6)	63	2.7	(0.51 - 8.3)
Total		306	20 ^a	(9.4 - 41)	128	8.4 ^a	(4.3 - 16)

GM: Williams' geometric mean; ^a Geometric mean.

The effect of the insecticide in preventing sandflies entering the bednet was assessed by comparing the percentage of sandflies that were captured inside the bednets (using as denominator the total number of caught sandflies). Surprisingly, this percentage was higher inside the ITN, 21.1% (95% C.I.: 14.0 – 28.2), compared with that in the untreated bednet, 8.5% (5.4 – 11.6). This difference was not statistically significant ($z = 1.84, p = 0.066$).

4.3.1.2 Landing rates and landing percentages

A total of 244 *L. longiflocosa* females were captured by human landing in the bedroom where the untreated bednet was used, compared to only 34 in the ITN room (Table 4.5); and the mean landing rate in the bedroom with the ITN was five times lower, 3.0 (2.0 - 4.4) f/p/3h, compared with the untreated bednet room, 15.0 (7.4 - 31) f/p/3h. The protective effect of the ITN, 80.6% (57.2 – 91.2), was highly significant ($z = 4.36; p < 0.001$). This protective effect was observed both inside and outside the net. The landing rate inside the ITN, 0.07 f/p/3h (1 female), was significantly lower than that

Table 4.5 Human landing rates of *L. longiflocosa* (females/person/3h) inside and outside ITN (lambda-cyhalothrin CS, 25 mg/m²) or untreated net (mesh 64 /cm²).

Site	Untreated bednet			Insecticide treated bednet			<i>p</i>
	No. fem.	GM	(95% C.I.)	No. fem.	GM	(95% C.I.)	
Inside the bednet	16	1.0	(0.18 - 2.5)	1	0.07	-	< 0.05
Outside the bednet	228	14 ^a	(6.9 - 29)	33	2.9 ^a	(2.0 - 4.3)	< 0.05
Total	244	15 ^a	(7.4 - 31)	34	3.0 ^a	(2.0 - 4.4)	< 0.001

GM: Williams' geometric mean; fem.:females; ^a Geometric mean.

inside the untreated bednet, 1.0 f/p/3h (16 females), indicating 94.0% (19.7 - 99.5) protection: $z = -2.13$, $p = 0.033$. Outside the ITN the landing rate was also significantly lower, 2.9 (2.0 – 4.3) f/p/3h, than that outside the untreated net, 14.0 (6.9 – 29.0) f/p/3h, indicating 79.7% (44.8 - 90.8) protection: $z = -4.25$, $p = 0.001$.

Regarding the percentage of *L. longiflocosa* females landing, all the analyzed percentages were lower in the room with the ITN (Table 4.6). With the ITN the percentage of all *L. longiflocosa* females that were captured on human bait, 29.1% (34 / 117), was significantly lower than that with the untreated net, 83.0% (244 / 294), indicating a 64.9% reduction in the percentage of biting females as a result of the use of the ITN : $z = -5.75$, $p < 0.001$. Similar results were obtained when the analysis was split according to the site of collection. Inside the ITN only 4.4% (1 / 23) of *L. longiflocosa* females were collected landing, while inside the untreated bednet the percentage of females collected landing increased to 69.7% (16 / 23): $X^2 = 18.29$, df: 1, $p < 0.001$. Outside the ITN 35.1% (33 / 94) of *L. longiflocosa* females were found landing, compared to 84.1% (228 / 271) outside the untreated bednet: $z = -4.79$, $p < 0.001$.

4.3.1.3 Mortality

Immediate mortality (a measure of the knockdown effect) was significantly higher in the room with the ITN, 68.8% (88 / 128), compared to only 5.6% (17 / 307) in the untreated bednet room: $z = 6.14$, $p < 0.001$ (Table 4.7). The same pattern was observed both inside and outside the nets. However, the immediate mortality of sandflies inside

Table 4.6 Percentage of *Lutzomyia longiflocosa* landing on human bait inside and outside ITN (lambda-cyhalothrin CS, 25 mg/m²) or untreated net (mesh 64 /cm²).

Site	Untreated bednet			Insecticide treated bednet			p
	fem. ^a landing / total fem.	%	(95% C.I.)	fem. landing / total fem.	%	(95% C.I.)	
Inside the bednet	16/23	69.7	-	1/23	4.4	-	< 0.001
Outside the bednet	228/271	84.1	(79.8 - 88.5)	33/94	35.1	(25.4 - 44.8)	< 0.001
Total	244/294	83.0	(78.7 - 87.3)	34/117	29.1	(20.9 - 37.3)	< 0.001

fem.: females

Table 4.7 Sandfly mortality inside bedrooms with ITN (lambda-cyhalothrin CS, 25 mg/m²) or untreated bednet (mesh 64 /cm²).

Mortality		Untreated bednet			Insecticide treated bednet			p
		No. dead / total sandflies	%	(95% C.I.)	No. dead/ total sandflies	%	(95% C.I.)	
Immediate	Inside the bednet	0/25	0	-	25/27	92.6	-	< 0.001
	Outside the bednet	17/279	6.1	(3.3 - 8.9)	63/101	62.4	(52.9 - 71.8)	< 0.001
	Total	17/304	5.6	(3.0 - 8.2)	88/128	68.8	(60.7 - 76.8)	< 0.001
24 h	Inside the bednet	15 ^a /25	60.0	(40.8 - 79.2)	27/27	100	-	< 0.001
	Outside the bednet	131 ^a /279	47.0	(41.1 - 52.8)	100/101	99.0	-	< 0.01
	Total	146/304	47.7	(42.1 - 53.3)	127/128	99.2	-	< 0.01

^a One female lost after catching was exclude from the analysis.

the ITN, 92.6% (25 / 27), was significantly higher than the mortality outside the same bednet, 62.4% (63 / 101): $X^2 = 7.7, p = 0.005$.

Mortality at 24 h was almost 100% (99.2%, 127 / 128) for sandflies caught in the bedroom with the ITN, compared with 47.7% (146 / 304) in the bedroom with the

untreated bednet: $z = 3.12$, $p = 0.002$. Similar patterns were obtained when the place of capture was taken into account (Table 4.7).

4.3.1.4 Percentage of females resting temporally on walls

The percentage of *L. longiflocosa* females found resting on the walls of the bedroom when the ITN was used was significantly lower, 2.6% (3 / 117), compared with the percentage resting on the walls of the bedroom with the untreated bednet, 8.2% (24 / 294): $z = -2.23$, $p = 0.026$.

4.3.2 Comparison of ITNs and house spraying for sandfly control

In the house spraying treatment, a median number of 3 bedrooms/ house ($q_{25} = 2$, $q_{75} = 3$; Min = 1, Max = 6) were sprayed; with a median sprayed area/ bedroom of 31.8 m² ($q_{25} = 24.1$, $q_{75} = 38.8$; Min = 19.7, Max = 58.9), and an actual median coverage of lambda-cyhalothrin of 21 mg/m² ($q_{25} = 18.5$, $q_{75} = 25.5$; Min = 15, Max = 32). With respect to the ITNs, a median number of 2 single and 1.5 double bednets were delivered per house, with a total median number of 4 bednets/ house ($q_{25} = 2.8$, $q_{75} = 5$; Min = 1, Max = 7), and a median coverage of insecticide/ bednet of 30.5 mg/m² (Min: 28, Max: 31), calculated from 4 impregnation batches (median number of bednets / batch = 24.5).

4.3.2.1 Sandfly abundance

The analysis was based on 48 houses (16 triplets) belonging to La Troja (12 triplets) and El Cedral (4 triplets) only, because for security reasons it was impossible to do the post-intervention sampling in Brasilia (7 triplets). Table 4.8 shows the total numbers and relative abundance of sandfly species caught during both pre-intervention and post-intervention sampling. In total 10,918 sandflies belonging to 8 species were collected. *L. longiflocosa* was the dominant species, accounting for 94.5% (10,316 / 10,918) of all captures. The second species, far behind, was *L. nuneztovari*, which accounted for 1.4% (153 / 10,918) of all captures. The other six species, 1.6% (171 / 10,918), were the same as those collected during the risk factor trial (Chapter 3). The remaining 2.5% (278 / 10,918) (which were not identified because of damage of fly structures used for

Table 4.8 Composition and relative abundance of sandflies caught by CDC light traps inside of the 48 houses included in the comparison of ITNs and house spraying, using lambda-cyhalothrin CS, 25 mg/m² (Treatment application was carried out in May 2001).

<i>Lutzomyia</i> species	Pre-intervention (January to March 2001)				Post-intervention (July 2001)				Total	
	♀ (%)	♂ (%)	total (%)	♀ (%)	♂ (%)	total (%)	♀ (%)	♂ (%)	total (%)	total (%)
<i>L. longiflocosa</i>	4316 (94.6)	475 (90.0)	4791 (94.0)	5295 (95.2)	230 (89.2)	5525 (94.9)	9611 (94.9)	705 (89.7)	10316 (94.5)	
<i>L. nuneztovari</i>	87 (1.91)	15 (2.84)	102 (2.00)	40 (0.72)	11 (4.26)	51 (0.88)	127 (1.25)	26 (3.31)	153 (1.40)	
<i>L. trinidadensis</i>	36 (0.79)	21 (3.98)	57 (1.12)	63 (1.13)	16 (6.20)	79 (1.36)	99 (0.98)	37 (4.71)	136 (1.25)	
<i>L. columbiana</i>	6 (0.13)	0 (0)	6 (0.12)	10 (0.18)	0 (0)	10 (0.17)	16 (0.16)	0 (0)	16 (0.15)	
<i>Helcocyrtomyia spp</i>	7 (0.15)	0 (0)	7 (0.14)	6 (0.11)	0 (0)	6 (0.10)	13 (0.13)	0 (0)	13 (0.12)	
<i>L. dubitans</i>	0 (0)	2 (0.38)	2 (0.04)	1 (0.02)	0 (0)	1 (0.02)	1 (0.01)	2 (0.25)	3 (0.03)	
<i>L. oresbia</i>	1 (0.02)	0 (0)	1 (0.02)	1 (0.02)	0 (0)	1 (0.02)	2 (0.02)	0 (0)	2 (0.02)	
<i>L. erwintonaldoi</i>	0 (0)	1 (0.19)	1 (0.02)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.13)	1 (0.01)	
Unidentified ^a	111 (2.43)	14 (2.65)	129 ^b (2.53)	147 (2.64)	1 (0.39)	149 ^c (2.56)	258 (2.55)	15 (1.91)	278 ^d (2.55)	
Total	4564	528	5096	5563	258	5822	10127	786	10918	

^a Damaged specimens ; ^b Included 4 specimens with unconfirmed sex; ^c Included 1 specimen with unconfirmed sex; ^d Included 5 specimens with unconfirmed sex.

identification) were considered as *L. longiflocosa*. The same pattern in species composition and relative abundance was observed in the two sampling periods.

In the pre-intervention samples, there was no significant difference in the GM abundance between treatments (ITNs, spraying, controls) either for total *L. longiflocosa* ($F_{(2, 45)} = 0.018$, $p = 0.982$), *L. longiflocosa* females ($F_{(2, 45)} = 0.009$, $p = 0.991$), *L. nuneztovari* females ($X^2_{(2)} = 3.61$, $p = 0.165$) or all sandfly females ($F_{(2, 45)} = 0.027$, $p = 0.973$).

In the analysis of the post-intervention samples, the mean abundance in houses with either of the two insecticide-based treatments appeared to be lower than that in the control houses (for all variables tested), with the lowest sandfly numbers caught in the houses with ITNs (Table 4.9). These apparent differences were not statistically significant for *L. longiflocosa* totals (analysis not shown) or females: female abundance, 50 f/LT/n for the control compared with 34 f/LT/n for sprayed houses, and 26 f/LT/n for houses with ITNs ($F_{(2, 44)} = 0.632$, $p = 0.536$). But the abundance of *L. nuneztovari* females in the ITN treatment, 0.2 f/LT/n, was significantly lower compared with the abundance in the control, 0.7 f/LT/n, ($z = -2.23$, $p = 0.026$). The same pattern was observed for *L. nuneztovari* totals with borderline significance: $z = -1.88$, $p = 0.06$. However, no significant differences were detected for *L. nuneztovari* abundance between houses with the two insecticide-based treatments ($z = -0.38$, $p = 0.701$).

4.3.2.2 Blood-fed females

Whilst average sandfly abundance in the pre-intervention sampling was similar for the three treatments (Table 4.9), as a result of the matching of triplets by sandfly abundance, the treatments were unbalanced by trophic status. There were statistically significant differences between treatments in the percentages of blood-fed females ($X^2_{(2)} = 140.25$, $p < 0.001$) and fully-fed females ($X^2_{(2)} = 32.74$, $p < 0.001$) (Table 4.10). The percentage of blood-fed females was significantly higher in the control treatment, 29% (27 - 31) compared with ITNs, 21% (19 - 24) ($z = -2.45$, $p = 0.014$), and house spraying 18% (16 - 20) ($z = -2.83$, $p = 0.005$). In contrast, the percentage of fully-fed females was significantly higher in the sprayed houses, 82% (78-87), compared with the control, 69% (65 - 73) ($z = -2.64$, $p = 0.008$) and the ITNs, 59% (53 - 66) ($z = -2.87$,

Table 4.9 Mean abundance indoors of *Lutzomyia longiflocosa*, *Lutzomyia nuneztovari* and total sandflies caught by CDC light traps, by treatment: ITNs, house spraying (both with lambda-cyhalothrin CS, 25 mg/m²) and controls (n = 16 per treatment).

Treatment	Species	Pre-intervention		Post-intervention	
		No.	GM (95% C.I.)	No.	GM (95% C.I.)
Control	Total <i>Lutzomyia</i>	2162	52 (29 - 94)	2626	54 (24 - 120)
	Total <i>Lutzomyia</i> females	1922	48 (27 - 85)	2550	52 (23 - 114)
	<i>L. longiflocosa</i>	2035	45 (24 - 84)	2469	50 (22 - 110)
	<i>L. longiflocosa</i> females	1861	43 (24 - 80)	2492	50 (23 - 111)
	<i>L. longiflocosa</i> males	226	2.4 (0.53 - 6.4)	63	1.9 (0.68 - 4.1)
	<i>L. nuneztovari</i> females	37	1.5 (0.62 - 2.7)	26	0.7 (0.08 - 1.6)
House spraying	Total <i>Lutzomyia</i>	1684	48 (27 - 85)	1777	42 (16 - 104)
	Total <i>Lutzomyia</i> females	1565	44 (25 - 78)	1645	37 (14 - 94)
	<i>L. longiflocosa</i>	1574	43 (24 - 76)	1663	35 (13 - 91)
	<i>L. longiflocosa</i> females	1518	42 (24 - 75)	1589	34 (13 - 86)
	<i>L. longiflocosa</i> males	91	2.2 (0.81 - 4.8)	116	3.8 (1.6 - 8.0)
	<i>L. nuneztovari</i> females	35	1.1 (0.35 - 2.3)	10	0.4 (0.03 - 0.82)
Insecticide treated bednets	Total <i>Lutzomyia</i>	1250	53 (34 - 82)	1419	28 (11 - 71)
	Total <i>Lutzomyia</i> females	1077	45 (28 - 71)	1368	26 (10 - 67)
	<i>L. longiflocosa</i>	1182	47 (28 - 77)	1393	27 (10 - 68)
	<i>L. longiflocosa</i> females	1048	42 (25 - 69)	1361	26 (9.8 - 66)
	<i>L. longiflocosa</i> males	158	4.0 (1.6 - 8.6)	51	1.8 (0.71 - 3.5)
	<i>L. nuneztovari</i> females	15	0.7 (0.3 - 1.3)	4	0.2 (0.03 - 0.40)

GM: Williams' geometric mean, sandflies/CDC trap/night.

p = 0.004). Because of these pre-intervention differences between treatments, the post-intervention analysis of blood-fed females, fully-fed females and their percentages

Table 4.10 Blood-fed females (all sandflies species) by treatment group: ITNs, house spraying (both with lambda-cyhalothrin CS, 25 mg/m²) and controls, during the pre-intervention trial (n = 16 houses per treatment).

Variable	Treatment							
	Control		House spraying		Insecticide treated bednet		Total	
	No.	GM or % (95% C.I.)	No.	GM or % (95% C.I.)	No.	GM or % (95% C.I.)	No.	GM or % (95% C.I.)
Total females	1922	48 (27 - 85)	1565	44 (25 - 78)	1077	45 (28 - 71)	4564	45 (34 - 60)
Blood-fed females	558	14 (6.9 - 29)	285	9.4 (4.6 - 18)	229	9.1 (5.0 - 16)	1072	11 (7.5 - 15)
Fully-fed females	376	10 (4.6 - 23)	234	7.8 (3.9 - 16)	136	6.4 (4.1 - 9.9)	746	8.0 (5.6 - 11)
% Blood-fed females	558/1922	29 ^a (27 - 31)	285/1565	18 ^b (16 - 20)	229/1077	21 ^b (19 - 24)	1072/4564	23 (22 - 25)
% Fully-fed females	376/547*	69 ^a (65 - 73)	234/285	82 ^b (78 - 87)	136/229	59 ^a (53 - 66)	746/1061*	70 (68 - 73)

^{a, b} Figures in the same row with different superscript letter presented statistical significant differences (*p* < 0.05). * Eleven missing data were excluded.

(Table 4.10) was adjusted by the pre-intervention data. The analysis was carried out only for *L. longiflocosa* as almost all blood-fed sandflies belonged to this species, 97.3% (588 / 604). Because no distinction by species was made for blood-fed females during the pre-intervention sampling, it was necessary to make the assumption (based on its overwhelming dominance) that all blood-fed and fully-fed females caught pre-intervention were *L. longiflocosa*.

The GM number of blood-fed females of *L. longiflocosa* was significantly reduced from 17 (7.7 - 34) f/LT/n in the control houses to 2.4 (2.0 - 4.8) f/LT/n in the sprayed houses ($z = -4.4, p < 0.001$) and to 1.0 (0.52 - 1.7) f/LT/n in the houses with ITNs ($z = -5.79, p < 0.001$), indicating 85.9% reduction due to house spraying and 94.1% due to the ITNs (Table 4.11). No significant difference was detected between the two treatments : $z = -1.41, p = 0.158$. The percentage of blood-fed females also decreased significantly from 20% (18 - 22) in the control houses to 4.3% (3.3 - 5.3) in the sprayed houses ($z = -5.74, p < 0.001$) and to 1.5% (0.89 - 2.2) in the houses with ITNs ($z = -5.16, p < 0.001$), indicating a 78.5% (69 - 88) proportional reduction due to house spraying and 92.5% (84.0 - 100) due to the treated nets.

The GM number of fully-fed females decreased significantly from 13 (5.6 - 27) f/LT/n in the control houses to 1.6 (0.70 - 3.1) f/LT/n in the sprayed houses ($z = -4.70, p < 0.001$) and to 0.62 (0.21 - 1.2) f/LT/n in the houses with ITNs ($z = -5.92, p < 0.001$), indicating 87.7% reduction due to house spraying and 95.3% due to the ITNs (Table 4.11). Finally, the percentage of fully-fed females decreased from 82.6% (79 - 86) in the control to 66.7% (C.I.: 47 - 87, unreliable because of low numbers) in the houses with ITNs (not significantly different: $z = -1.24, p = 0.216$) and to 60.3% (49 - 72) in the sprayed houses (borderline significance: $z = -1.96, p = 0.05$). For sprayed houses, this indicates a 27.7% (13 - 42) proportional reduction in the percentage of fully-fed females.

Although, in general sandflies appeared to have more feeding success in sprayed houses than in houses with nets, no significant differences between the two treatments were detected for any of the four variables tested: GM blood-fed females ($z = -1.40, p = 0.158$), percentage of blood-fed females ($z = -1.11, p = 0.265$); GM number of fully-fed females ($z = -1.37, p = 0.172$) or percentage of fully-fed females ($z = 0.13, p = 0.897$).

Table 4.11 Blood-fed females of *Lutzomyia longiflocosa* by treatment group: ITNs, house spraying (both with lambda-cyhalothrin CS, 25 mg/m²) and controls, during the post-intervention trial (n = 16 houses per treatment).

Variable	Treatment							
	Control		House spraying		Insecticide treated bednets		Total	
	No.	GM or % (95% C.I.)	No.	GM or % (95% C.I.)	No.	GM or % (95% C.I.)	No.	GM or % (95% C.I.)
Total females	2492	50 ^a (23 - 111)	1589	34 ^a (13 - 86)	1361	26 ^a (9.8 - 66)	5442	35 (22 - 57)
Blood-fed females	499	17 ^a (7.7 - 34)	68	2.4 ^b (2.0 - 4.8)	21	1.0 ^b (0.52 - 1.7)	588	3.9 (2.3 - 6.3)
Fully-fed females	412	13 ^a (5.6 - 27)	41	1.6 ^b (0.70 - 3.1)	14	0.62 ^b (0.21 - 1.2)	467	2.9 (1.7 - 4.7)
% Blood-fed females	499/2492	20.0 ^a (18 - 22)	68/1589	4.3 ^b (3.3 - 5.3)	21/1361	1.5 ^b (0.89 - 2.2)	588/5442	10.8 (10 - 12)
% Fully-fed females	412/499	82.6 ^a (79 - 86)	41/68	60.3 ^b (49 - 72)	14/21	66.7 ^{a,b} (47 - 87)	467/588	79.4 (76 - 83)

Figures in the same row with different superscript letter presented significant differences (*p* < 0.05).

4.3.2.3 Proportion of females with human blood or HBI

During the post-intervention sampling, 604 blood-fed females were collected, of which 97.3% (588) were *L. longiflocosa*. The remaining 2.7% (16), included thirteen females of *L. nuneztovari*, two *Helcocyrtomyia* sp. and one *L. columbiana*. Four females which were lost during the dissection in the laboratory were excluded from the analysis (Table 4.12). The precipitin ring test for identification of human blood was carried out on half-fed plus fully-fed females only. In total 88.6% (521 / 588) of blood-fed females of *L. longiflocosa* were tested, with an overall HBI of 0.793 (413 / 521). In addition, 12 (of 13) specimens *L. nuneztovari* and the only one of *L. columbiana* were also positive for human blood. The analysis was carried out on *L. longiflocosa* only as the low numbers of the other species precluded any analysis.

Pre-intervention data were not collected from all houses in the trial, but comparison of the pre-intervention HBI for *L. longiflocosa* in twelve houses (four triplets), where suitable samples for the precipitin ring test were collected, showed no statistical difference between the control group, 0.714 (10 / 14) compared with the sprayed house group, 0.771 (27 / 35) (Fisher's exact test, $p = 0.721$), or the ITN house group, 0.563 (9 / 16) ($X^2 = 0.23$, $p = 0.630$).

Post-intervention data showed that the HBI decreased significantly from 0.845 (0.812 - 0.879) in the control houses to 0.500 (0.367 - 0.633) in the sprayed houses ($z = -2.60$, $p = 0.009$) and to 0.267 (C.I.: 0.153 - 0.381, unreliable because of small numbers) in the houses with ITNs ($z = -3.02$, $p = 0.003$) (Table 4.13), reflecting a proportional reduction in the HBI of 40.8% (24.6 - 57.0) in the sprayed houses and 68.4% (C.I.: 35 - 80, unreliable because of low numbers) in the houses with ITNs. The apparent difference between the two treatments with insecticide was not significant ($z = -1.38$, $p = 0.168$).

Finally, significant effects of both blood condition and amount on the results of the precipitin test were detected. The proportion of positive human blood identifications was significantly higher in females with fresh blood (bright blood), 0.858 (205 / 239, C.I.: 0.813 - 0.902), compared with females with digested blood (brown or black), 0.738 (208 / 282, C.I.: 0.686 - 0.789) ($z = -3.60$, $p < 0.001$). Similarly, the proportion of

Table 4.11 Fed female sandflies caught indoors by CDC light traps during the post-intervention trial, according to blood condition and amount.

Blood amount species		Blood condition												Total	
		Fresh						Digested							
		Few (25%)		Half (50%)		Full (>75%)		Few (25%)		Half (50%)		Full (>75%)			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>L. longiflocosa</i>		16	100	25	100	216	98.6	47	94.0	33	97.1	251	96.5	588	97.4
<i>L. nuneztovari</i>		0		0		2	0.9	1	2.0	1	2.9	9	3.5	13	2.2
<i>L. columbiana</i>		0		0		1	0.5	0		0		0		1	0.2
<i>Helcocyrtomyia</i> sp.		0		0		0		2	4.0	0		0		2	0.3
Total		16		25		219		50		34		260		604	

positive human blood identifications was significantly higher in fully-fed females, 0.811 (377 / 463, C.I.: 0.776 - 0.847), compared with half-fed females, 0.621 (36 / 58, C.I.: 0.496 - 0.746) ($z = 2.54, p = 0.011$) (Table 4.10). After adjusting for these two variables, blood amount and condition, the statistical differences in HBI between the three house groups remained ($X^2_{(2)} = 59.44, p < 0.001$).

4.3.2.4 Perception of householders on effectiveness of control measures and side effects

All the 16 householders (i.e. the heads of the household) of the houses provided with ITNs were interviewed about their opinions on the effectiveness of this measure; 69% (11 / 16) of householders in sprayed houses were similarly interviewed.

Regarding the perception of effectiveness, in sprayed houses, 73% (8 / 11) of householders considered that this measure had some benefit in the control of sandflies and other bugs; while the remaining 27% (3 / 11) said that the nuisance caused by sandflies continued after treatment. From the percentage who reported some benefit from spraying, 50% (4 / 8) said that the effectiveness of the insecticide was lost quickly and that by the time of the interview (three months after treatment) the sandflies were a

Table 4.13 Proportion of *Lutzomyia longiflocosa* females with human blood detected by the precipitin ring test, by blood condition and amount according to the treatment group: ITNs, house spraying (both with lambda-cyhalothrin CS, 25 mg/m²) and controls, during the post-intervention trial (n = 16 per treatment).

Blood		Treatment					
		Control			House spraying		
Condition	Amount	HU+/total fem.*	Proportion	(95% C.I.)	HU+/total fem.	Proportion	(95% C.I.)
Fresh	Full	173/182	0.951	-	13/24	0.542	-
	Half	8/12	0.667	-	8/12	0.667	-
	Subtotal	181/194	0.933	(0.898 - 0.968)	21/36	0.583	-
Digested	Full	181/227	0.797	(0.745- 0.850)	6/17	0.353	-
	Half	20/31	0.645	(0.477 - 0.813)	0/1	0	-
	Subtotal	201/258	0.778	(0.729 - 0.830)	6/18	0.333	-
Total	Full	354/409	0.866	(0.832 - 0.898)	19/41	0.463	-
	Half	28/43	0.651	-	8/13	0.625	-
	Total	382/452	0.845 ^a	(0.812 - 0.879)	27/54	0.500 ^b	(0.367 - 0.633)
					4/15	0.267 ^b	(0.153 - 0.381)

Figures with different superscript letters presented significantly differences ($p < 0.05$); ^a HU+: positive females for human blood, total fem.: total number of tested females.

nuisance in their houses again. In contrast, all householders with ITNs considered that the treated bednets were useful to control sandflies, and 44% (7 / 16) reported that sandflies and/or bugs that made contact with the bednet were killed.

With respect to side effects, in the sprayed houses there was only one report of nasal irritation. In contrast, for ITN treatment, 44% (7 / 16) of householders reported some short term effect, such as irritancy, sneezing and rhinorrhoea, in some of the family members, during a few days after they begun to use the bednet. From this percentage, 57% (4 / 7) reported throat or nasal irritation and/or sneezing, while 29% (2 / 7) reported eyes, nasal or throat irritation only, and one householder reported rhinorrhoea, in addition to nasal irritation. Finally, in relation to the comfort of the bednets, all ten householders who were asked if the bednets were comfortable to sleep under responded positively. Some of them (3 / 10) reported gratefully that the bednets protected from the cold during the night.

4.3.3 Validation of use of CDC light traps as substitute for human landing

The analysis was carried out on 13 triplets of houses (39 houses) used in the comparison between ITNs and house spraying (section 4.3.2). The initial planned sample size of 16 triplets was not achieved because it was impossible to sample three houses by human landing, so the corresponding three triplets were dropped from the study. The 13 triplets were well matched by pre-intervention sandfly abundance (Table 4.14)

A total of 738 sandflies (all females) were caught by human landing, of which 98.6% (728 / 738) were *L. longiflocosa*. The remaining 1.4% corresponded to four *L. nuneztovari*, one unidentified species of *Helcocyrtomyia* and five unidentified specimens. With CDC light traps 4,725 sandflies were caught, again with an overwhelming dominance of *L. longiflocosa*, accounting for 95.1% (4,495 / 4,725), with 1.0% (48 / 4,725) of *L. nuneztovari*, 1.0% (49 / 4,725) including four species (*L. trinidadensis*, *L. columbiana*, *L. dabitans* and *Helcocyrtomyia sp*), and 2.8% (133 / 4,725) of unidentified females. For *L. longiflocosa* the catches were strongly female biased: 95.7% (4,303 / 4,495) were females. The analysis was carried out only on *L. longiflocosa* females, with unidentified females considered as *L. longiflocosa*, as has been assumed in the other studies of this thesis.

Table 4.14 Catches by human landing (HLC), CDC light traps (LTC) and their corresponding ratios for *Lutzomyia longiflocosa* indoors, by treatment group: ITNs, house spraying (both with lambda-cyhalothrin CS, 25 mg/m²) and controls (n= 13 houses per treatment).

Treatment	Method									
	LTC					HLC				
	(females / trap / night)					(females/ person / 2.5 h)				
	Pre-intervention		Post-intervention			Post-intervention				
	fem.	GM	(95% C.I.)	fem.	GM	(95% C.I.)	fem.	GM	(95% C.I.)	GM ratio ^a LTC: HLC (95% C.I.)
Control	1803	52	(25 - 105)	2406	65	(28 - 150)	220	5.8	(2.1 - 14)	9.7 (3.4 - 28)
House spraying	1470	51	(27 - 98)	1282	37	(13 - 101)	422	5.9	(1.2 - 21)	5.4 (1.8 - 16)
Insecticide treated bednet	988	48	(26 - 88)	748	23	(8 - 63)	91	2.0 ^b	(0.4 - 5.8)	7.8 (3.4 - 18)
Total	4261	50	(36 - 71)	4436	38	(22 - 64)	733	4.2	(2.2 - 7.7)	7.4 (4.4 - 13)

GM : Williams' geometric mean; fem.: females; ^a GM ratio: Antilog of the mean of ln [(LTC + 1) / (HLC + 1)]; ^b Catches outside of insecticide treated bednets.

4.3.3.1 Correlation between the two sampling methods

A significant positive correlation was found between the human landing catches and the CDC trap catches ($r = 0.468$, $t = 3.22$, $df = 37$, $p = 0.003$) (Figure 4.9). The \ln ratio for the whole data set was 2. Therefore the GM ratio ($\exp(\ln \text{ ratio}) = \exp(2)$) for the whole data set was 7.4 (4.4 - 13) (Table 4.14). This means that on average, one CDC light trap (18:00 - 7:00 h) caught 7.4 times the number of *L. longiflocosa* females collected by a single human bait (19:00 - 21:30 h) in the same house.

4.3.3.2 Effect of insecticide treated bednets and house spraying on the relative efficiency of the sampling methods

The possible effect of treatments on the sampling methods was tested in two ways:

(1) Comparison of the mean log ratios, $\ln[\{\text{LTC} + 1\} / \{\text{HLC} + 1\}]$ (shown as GM ratios), between treatments. The control houses showed the highest GM ratio, 9.7 (3.4 - 28), followed by the houses with ITNs, 7.8 (3.4 - 18). The lowest GM ratio was found in the sprayed houses, 5.4 (1.8 - 16) (Table 4.14). Nevertheless, there was no statistical difference between treatments ($F_{(2, 24)} = 0.615$, $p = 0.548$). On the other hand, as expected, a significant proportion of the variance in the log ratios was explained by differences between triplets ($F_{(12, 26)} = 2.52$, $p = 0.024$). This suggests that the relative efficiency of the two sampling methods could depend on variation in sandfly abundance between triplets.

(2) Testing for any effect of sandfly abundance on the relative sampling efficiency of the methods. Figure 4.10 shows how the difference between the two methods $[\ln\{\text{LTC} + 1\} - \ln\{\text{HLC} + 1\}]$ varies with a joint estimate of sandfly abundance $[\{\ln(\text{LTC} + 1) + \ln(\text{HLC} + 1)\} / 2]$. There was no significant correlation ($r = 0.014$, $t = 0.086$, $df = 37$, $p = 0.466$) between the log ratios and the log sandfly abundance.

4.3.3.3 Comparison between treatments by human landing catches

Although it was not the objective of this section to compare the treatments, the data do provide a direct measure of the impact of treatment on human landing rates. The GM

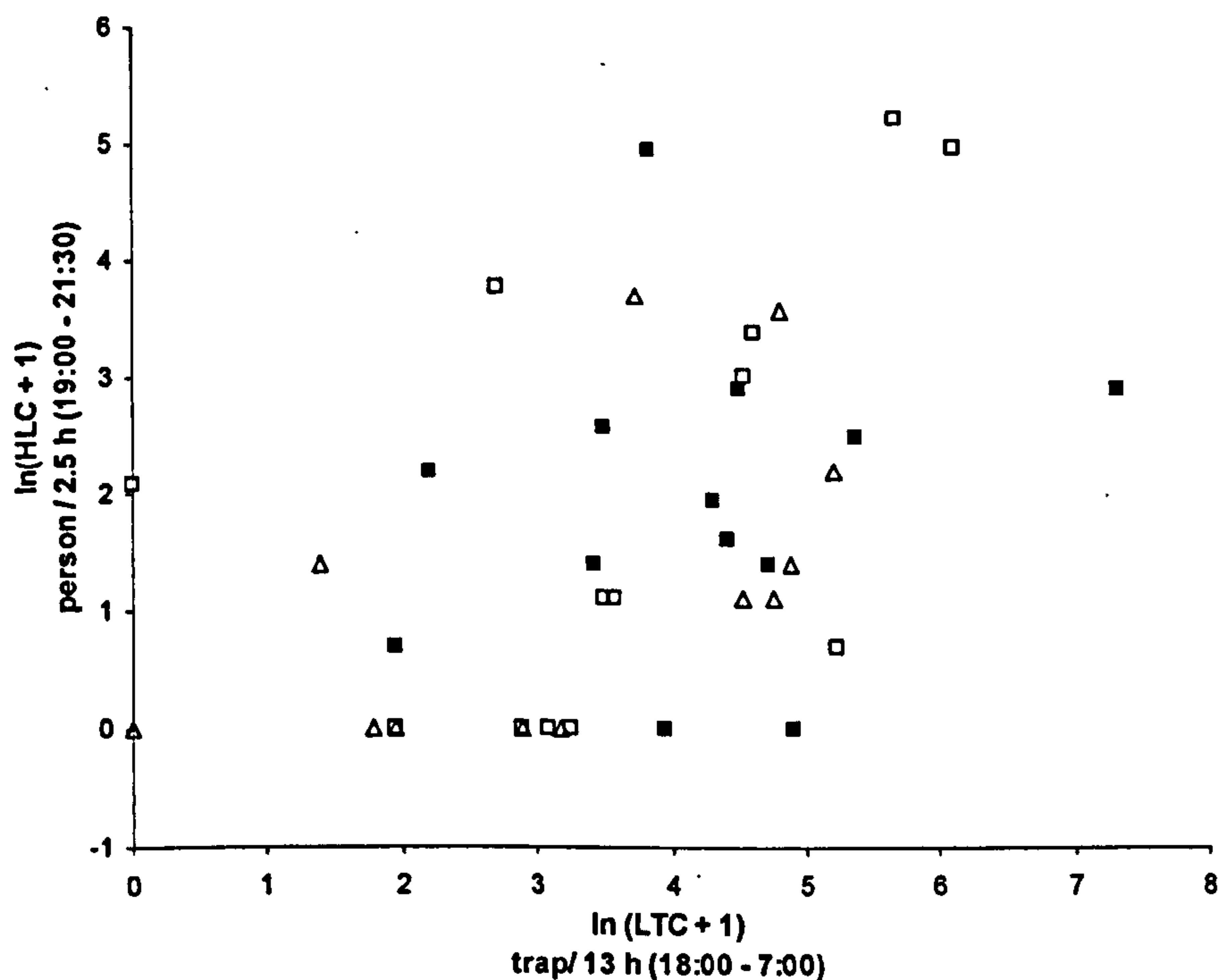


Figure 4.9 The relationship between human landing catches (HLC) and CDC light traps catches (LTC) of *Lutzomyia longiflocosa* females in houses with ITNs (Δ), sprayed houses (\blacksquare), and controls (\square). Each point represents a pair of catches on consecutive nights in the same house.

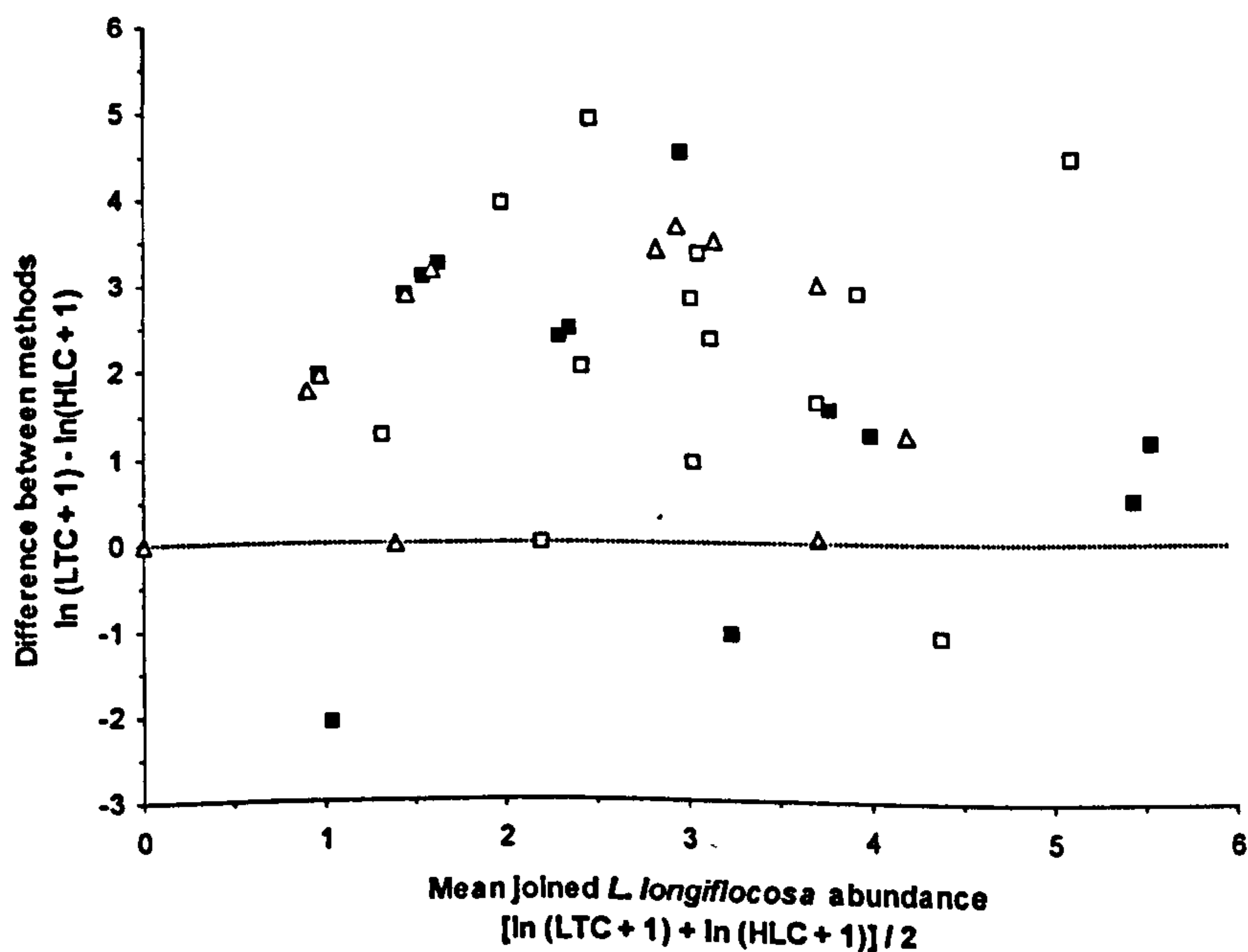


Figure 4.10 The relationship of the differences between methods $[\ln\{\text{LTC} + 1\} - \ln\{\text{HLC} + 1\}]$, which is the same as \ln ratio $\ln\{\text{LTC} + 1\} / \{\text{HLC} + 1\}$, against the mean of $\ln[\text{LTC} + 1]$ and $\ln[\text{HLC} + 1]$ catches for *Lutzomyia longiflocosa* females by treatment, using the same data as in Figure 4.9. Symbols as in Figure 4.9.

number of *L. longiflocosa* females caught by human landing in the post-intervention sampling was apparently lower in the houses with ITNs, 2.0 (0.4 - 5.8) f/p/2.5 h compared with the controls, 5.8 (2.1 - 14) f/p/2.5 h, and the sprayed houses, 5.9 (1.2 - 21) f/p/2.5 h, which presented apparently similar landing rates (Table 4.14). Although, in general no significant differences were detected between treatments ($F_{(2, 24)} = 2.42$, $p = 0.108$), the lower human landing rate in houses with ITNs was borderline non-significant when compared with either the control houses ($z = 1.88$, $p = 0.06$) or the sprayed houses ($z = 1.93$, $p = 0.054$). This reduction in landing rate in the houses with ITNs is consistent with the earlier findings of an apparent reduction in abundance of *L. longiflocosa* females, and a significant reduction in blood-fed females, fully-fed females and in percentage of blood-fed females. In contrast, the impact of house spraying on sandfly biting rates indoors is less clear, as the absence of any observed effect in this study does not match with the apparent reduction in feeding success detected in the sandflies collected by light traps in the same houses.

4.3.4 Residual effect of insecticide on bednets and walls

4.3.4.1 Bioassays on insecticide treated bednets

A total of fourteen assays were carried out involving 234 wild sandflies, mostly *L. longiflocosa*, 90.6% (212 / 234), and mostly females, 93.9% (199 / 212). The remaining sandflies (9.4%) were female *L. nuneztovari* (15) and *L. columbiana* (7). Hence, it can be assumed that the results are representative for *L. longiflocosa* females.

The lambda-cyhalothrin CS, 25 mg/m² treated bednets kept their initial high insecticidal effectiveness 4 months after treatment (Table 4.15). The mean 24 h mortality 4 months after treatment, 96.6% (Min: 87.5%, Max: 100%) was not significantly different from the mortality caused by freshly treated bednets 98.9% (Min: 92.3%, Max: 100%) (Fisher's exact test, $p = 0.621$). Both mortalities were significantly higher, for freshly treated bednets ($\chi^2 = 51.55$, $p < 0.001$); and for bednets after 4 months ($\chi^2 = 45.42$, $p < 0.001$), compared with the mortality amongst the controls (untreated bednets): 47.4% (Min: 37.5%, Max: 60%). Mortality recorded 1 h after treatment, confirmed the high effectiveness of the ITN after 4 months, with 83.1% (Min: 66.7%, Max: 100%)

Table 4.15 Residual effect of lambda-cyhalothrin CS, 25 mg/m², applied on polyester bednets (0.7 mm mesh size), on mortality of wild sandflies (90.6% *Lutzomyia longiflocosa*, where females were 93.9%) following 3 minutes exposure.

			Time after exposure					
Treatment	Bioassay	Total san.	0 h		1 h		24 h	
			No. dead	%	No. dead	%	No. dead	%
Untreated bednet	1	13	0	0	0	0	7	53.8
	2	16	0	0	0	0	6	37.5
	3	15	0	0	0	0	9	60.0
	4	13	0	0	0	0	5	38.5
	total	57	0	0 ^a	0	0 ^a	27	47.4 ^a
Freshly treated bednet	1	14	0	0	13	92.9	14	100
	2	16	2	12.5	16	100	16	100
	3	15	0	0	14	93.3	15	100
	4	15	0	0	15	100	15	100
	5	15	0	0	15	100	15	100
	6	13	0	0	12	92.3	12	92.3
	total	88	2	2.3 ^a	85	96.6 ^b	87	98.9 ^b
Treated bednet after 4 months of use	1	18	0	0	12	66.7	18	100
	2	17	1	5.9	12	70.6	17	100
	3	11	0	0	10	90.9	10	90.9
	4	14	0	0	14	100	14	100
	5	13	0	0	12	92.3	13	100
	6	16	0	0	14	87.5	14	87.5
	total	89	1	1.1 ^a	74	83.1 ^c	86	96.6 ^b

Values with different superscript by column are statistically different, *p* < 0.001; san.: Sandflies.

mortality. However, this was significantly lower than the 1 hr mortality caused by freshly treated bednets, 96.6% (Min: 92.3%, Max: 100%) ($X^2 = 7.35$, $p = 0.007$). There was no 1 h mortality amongst the control group. Mortality rates immediately after exposure were only 2.3% (Min: 0%, Max: 12.5%) and 1.1% (Min: 0%, Max: 5.9%) for the freshly treated nets and those nets after 4 months of treatment, respectively (no significant difference compared with untreated nets : Fisher's exact test, $p = 0.520$ for freshly treated bednets, and $p = 1.000$ for bednets 4 months after treatment) .

4.3.4.2 Bioassays on sprayed walls

Eighteen assays were carried out on sprayed walls, involving 297 wild sandflies, mostly *L. longiflocosa*, 94.6% (281 / 297), and mostly females, 84.7% (238 / 281). The remaining sandflies (5.4%) were female *L. nuneztovari* (fourteen), *L. columbiana* (one) and an unidentified species (one). Hence, it can be assumed that the results are representative for *L. longiflocosa* females.

The results showed that indoors spraying with Lambda-cyhalothrin CS, 25 mg/m², on "bahareque" plastered and painted walls decreased in effectiveness 4 months after treatment (Table 4.16). The mean 24 h mortality 4 months after treatment was high, 90.1% (Min: 66.7%, Max: 100%), but significantly lower compared with mortality in freshly treated walls where all tested sandflies were killed (Fisher's exact test, $p < 0.001$). Both mortalities were significantly higher ($X^2 = 57.85$, $p < 0.001$, for freshly treated walls; and $X^2 = 26.46$, $p < 0.001$, for walls 4 months after spraying) compared with the mortality amongst controls (unsprayed walls), 55.6% (Min: 27.8%, Max: 93.8%). Mortality recorded 1 h after exposure, confirmed the drop in effectiveness of the spraying after 4 months, with 72.5% (Min: 0%, Max: 100%) mortality, compared to 100% caused by freshly treated walls ($X^2 = 31.2$, $p < 0.001$). Mean 1hr mortality in the control group was 3.0% (Min: 0%, Max: 13.3%). A 4-month decrease in effectiveness was also evident immediately after exposure when mortality was only 30.8% (Min: 0%, Max: 75.0%) 4 months after treatment, compared to 97.2% (Min: 84.2%, Max: 100%), for freshly treated walls ($X^2 = 94.68$, $p < 0.001$) Mortality in the control group immediately after exposure was only 1.0% (Min: 0, Max: 6.3%).

Table 4.16 Residual effect of lambda-cyhalothrin CS, 25 mg/m², applied indoors on "bahareque" plastered and painted walls, on mortality of wild sandflies (94.6% *Lutzomyia longiflocosa*, where females were 84.7%) following 1 hour exposure.

Treatment			Time after exposure					
			0 h		1 h		24 h	
			Bioassay	Total san.	No. dead	%	No. dead	%
Unsprayed walls	1	18	0	0	0	0	5	27.8
	2	18	0	0	0	0	10	55.6
	3	15	0	0	0	0	12	80.0
	4	16	1	6.3	1	6.3	15	93.8
	5	15	0	0	2	13.3	6	40.0
	6	17	0	0	0	0	7	41.2
	Total	99	1	1.0 ^a	3	3.0 ^a	55	55.6 ^a
Freshly sprayed walls (3 days)	1	21	21	100	21	100	21	100
	2	19	19	100	19	100	19	100
	3	18	18	100	18	100	18	100
	4	14	14	100	14	100	14	100
	5	19	16	84.2	19	100	19	100
	6	16	16	100	16	100	16	100
	Total	107	104	97.2 ^b	107	100 ^b	107	100 ^b
Walls 4 months after spraying	1	15	6	40.0	10	66.7	10	66.7
	2	14	7	50.0	14	100	14	100
	3	12	0	0	0	0	10	83.3
	4	16	12	75.0	15	93.8	15	93.8
	5	18	3	16.7	14	77.8	18	100
	6	16	0	0	13	81.3	15	93.8
	Total	91	28	30.8 ^c	66	72.5 ^c	82	90.1 ^c

Values with different superscript by column are statistically different, *p* < 0.001; san.: Sandflies.

4.4 DISCUSSION

4.4.1 Summary

Experimental trials showed that ITNs reduced human landing rates by *L. longiflocosa* both inside and outside nets. There was no convincing evidence that the insecticide treatment on the nets caused any significant excito-repellency effects, nor any diversion of sandflies to feed more on people outside the nets but in the same room. Biting inside nets was reduced by treatment even if using wide mesh nets – not by preventing entry but by causing immediate mortality for sandflies that have passed through a net (i.e. knockdown effect). But even untreated wide mesh nets seemed to provide significant protection as compared to people outside the nets. Effectiveness of insecticide on nets was maintained for at least 4 months (as shown by bioassays); and outside-net landing rates were also still significantly lower in rooms with nets 4 months post-treatment. Inside-net landing rates were not tested directly at 4 months, but light trap catches in rooms with people sleeping under nets 4 months post-treatment contained less sandflies, a lower proportion of bloodfeds, smaller blood meals, and a lower HBI than in control houses. The ratio of light trap catches to human landing rates outside nets was the same as in control houses demonstrating that the observed reduction in sandfly numbers collected in rooms with ITNs (as measured either by light traps or by a combination of direct search and human catches) reflects a true difference, and is the principal reason why treated nets appear to provide protection to people outside them. The lower numbers may not necessarily be explained because less sandflies enter these houses, but because a significant proportion get knocked down after making contact with an ITN, and before taking a bloodmeal or entering a light trap.

The effect of house spraying on sandfly biting rates was less clear-cut. The effectiveness of the insecticide significantly dropped by 4 months, but still caused considerable mortality. Light trap catches at 4 months found less sandflies, a lower percent of bloodfeds, smaller bloodmeals, and a lower HBI than in control houses. However, there was some suggestion that light traps are less effective in sprayed houses as the ratio of light trap catches to human landing rate catches in sprayed houses was considerably less than in control houses. Furthermore a direct comparison of human landing catches in sprayed vs control houses found absolutely no difference. Hence it is unclear the extent (if any) to which house spraying protected people. As the trial was designed to

designed to investigate protection at the household level, the results do not preclude the possibility that mass spraying of all houses could significantly reduce the sandfly population and so reduce risk. A detailed interpretation of all the results follows.

4.4.2 Effect of insecticide treatment on bednet efficacy

This study was designed to measure the impact of insecticide treatment of bednets under experimental conditions. The bednets used had just been treated with lambda-cyhalothrin prior to the study.

4.4.2.1 Female abundance

The significantly lower "abundance" of *L. longiflocosa* females in the bedroom with the ITNs, 7.9 f/p/3h, compared with the untreated bednet, 18.8 f/p/3h ($p = 0.033$) could be explained, in part, by limitations of the sampling method. This is because with the ITN, some sandflies that were knocked down could have been lying outside the collection area surrounding the bednet (30 cm around the bednet), and hence were not accounted for. This is a more parsimonious explanation than assuming a deterrent or excito-repellent effect, as there was no evidence that insecticide treatment of nets prevented sandflies passing through the wide mesh: with 21.1% of collected females inside the ITN compared to 8.5% inside the untreated bednet.

Lambda-cyhalothrin works by direct contact and has a low vapour pressure (Miller *et al.*, 1991). Moreover, the microencapsulate (CS) formulation is characterized by a reduced release of the active ingredient (Palchick 1996). These factors would contribute to a marked reduction or absence of any excito-repellent or deterrent effect of the insecticide (up to 20 mg /m²) as reported for the mosquito *Anopheles gambiae* in experimental huts in Dar es Salaam (Miller *et al.*, 1999) and Muheza (Maxwell *et al.*, 1999) in Tanzania. Nevertheless, deterrent and excito-repellent effects could vary widely as they depend on the interaction of several variables, e.g. type of insecticide, formulation, doses and the vector species, or even strain, to be targeted. For example, also in experimental huts, deterrent and excito-repellent effects of lambda-cyhalothrin CS (10 and 15 mg/m²) were detected for *A. gambiae* and *A. funestus* in M'be Valley, Cote d' Ivoire (Darriet *et al.*, 1999). Bioassay comparisons of species showed that

lambda-cyhalothrin (25 mg/m²) was relatively nonirritant to *A. gambiae*, while it causes high irritancy to *Culex quinquefasciatus* (Miller and Gibson 1994).

With sandflies, lack of excito-repellent or deterrent effect has been also suggested, but with other pyrethroids. In Valle del Cauca, Colombia, there was no significant differences in the abundance of *L. youngi* and other anthropophilic sandflies species inside control houses (collected by a combination of direct search and human landing) as compared with houses with deltamethrin (26 mg/m²) treated bednets or curtains (both with mesh 64 /cm²) (Alexander *et al.*, 1995c). In Khartoum, Sudan, the indoor abundance of *P. papatasi* (measured by sticky traps) was also not significantly different in houses fitted with permethrin (500, 1000, or 1500 mg/m²) treated curtains (mesh size 1.1 mm) and houses with or without untreated curtains (Elnaiem *et al.*, 1999a).

4.4.2.2 Human landing rates

Treatment of the bednet reduced the landing rate of *L. longiflocosa* inside the net by 94%. This reduction is comparable to results of several experimental and field studies with mosquitoes (Miller *et al.*, 1991; Curtis *et al.*, 1996), and consistent with the few previous sandfly studies that reported comparisons of the impact of treated or untreated materials. In Sudan, insecticide (lambda-cyhalothrin, 10 mg/m²) treated bednets (156 /sq. inch mesh = 25 /cm² mesh, approximately) were compared (over 12 nights) with untreated bednets outdoors. The human landing rate of *P. orientalis* inside the nets dropped from 6.9 f/p/night in an untreated bednet to "zero" inside an ITN (Elnaiem *et al.*, 1999b). In Venezuela, where the biting activity of *L. spinicrassa* females was recorded in a tent divided into two compartments, each with an untreated or treated (deltamethrin 1000 mg/m²) curtain as door, the percentage of sandflies biting was reduced from 13.3%, in the compartment with the untreated curtain, to 0.016% in the compartment with the insecticide treated curtain, indicating a reduction of 99.9% (Perruolo 1995). Finally, in Colombia, the indoor landing rate of *L. youngi* (over 26 nights) was reduced by 62% from 0.69 f/p/h inside an untreated bednet to 0.26 f/p/h inside a deltamethrin EC (26 mg/m²) treated bednet (64 mesh /cm²) (Alexander *et al.*, 1995c).

Whilst the reduction in sandfly landing rate due to pyrethroid treatment of nets or curtains is clear, the mechanism is less well understood. It is possible that the insecticide

affects the mechanisms involved in the feeding behavior (and as a result the insect fails to bite) and/or in the blood intake. In the present study the reduction in the landing rate (and percentages of landing) seem to result principally from the knockdown effect (immediate mortality). Inside the ITN almost all the sandflies were knocked down before biting. Only a single female, of two that were found alive, was able to bite. There is also evidence that blood intake was affected as the size of bloodmeals taken in insecticide treated houses (bednets or spraying) was lower than in control houses (see below).

The 80% reduction in the landing rate outside the ITN compared with the untreated bednet indicates that treatment did not result in diversion of sandflies to unprotected persons outside the ITN (i.e. due to some excito-repellence effect). This is important because it means that people who sleep outside an ITN would also benefit from it. The same lack of diversion effect due to insecticide treatment of nets has been demonstrated for malaria vectors (Lines *et al.*, 1987). For sandflies the only previous study (Alexander *et al.*, 1995c) to address this issue compared indoor landing rates on a person outside a deltamethrin impregnated (26 mg/m^2) bednet (1.9 s/p/h) with the landing rate on a person in an unprotected room (3.3 s/p/h), demonstrating 42% protection. The explanation for protection accrued by people outside an ITN appears to be the knockdown effect, as the overall difference in the percentage of females landing outside the untreated (84.1%) and treated (35%) bednets was no longer significant ($z = 0.49$, $p = 0.626$) when the knocked down sandflies were excluded from the analysis: 89.4% (228 / 255) in the untreated bednet versus 89.2% (33 / 37) in the ITN.

Another remarkable finding was the protective effect of the untreated bednet, despite the relatively large mesh (64 /cm^2), with landing rates inside the untreated bednet, 1.0 (0.18 - 2.5) f/p/3h, compared with the landing rate outside of the same bednet, 14 (6.9 - 29) f/p/3h. The effect of untreated bednets on mosquitoes is widely appreciated (Curtis *et al.*, 1996), but generally it has been believed that only narrow mesh nets could protect against relatively small sandflies unless treated with insecticide. Because the result is unexpected, it should be interpreted with caution as a host choice effect (i.e. sandflies could prefer to bite the unprotected person in the same room instead of attempting to bite the person protected under the bednet) should not be discarded as the cause of the lower landing rate inside the untreated bednet. It is necessary to test the untreated

bednet in the scenario where all the persons in a room sleep under the bednet to confirm if the landing rates inside the bednet remains lower than in a room with no bednets. Previous studies indicate this may be possible. In the Sudan, the outdoor human landing rate of *P. orientalis* inside untreated nets with mesh equivalent to 25 /cm² (6.9 f/p/night) was significantly lower than that on a person without a bednet (32.0 f/p/night) (Elnaiem *et al.*, 1999b). In Nepal, a case control study identified the use of untreated bednets (mesh size not given, but mentioned as relatively large) as a protective factor against VL transmitted by *P. argentipes* (Bern *et al.*, 2000). A similar protective effect from untreated nets was also reported from a risk factor analyses of VL in Bangladesh (Bern, 2005). In contrast, a recent study carried out in Turkey showed that untreated wide mesh bednets (mesh mistakenly reported as 156 /cm², but appear to have been in reality 25/cm²) did not reduce incidence of CL transmitted by *P. sergenti* and *P. papatasi*. The authors suggested that the wide mesh failed to prevent sandflies entering the bednets and biting people inside (Alten *et al.*, 2003).

4.4.2.3 Mortality

The high mortality, both immediate (68.8%) and at 24 hours (99.2%), found with the ITNs confirm the high insecticidal effect of lambda-cyhalothrin compared with other pyrethroids (as observed in experimental huts for several mosquito species such as *A. gambiae* (Curtis *et al.*, 1996; Miller *et al.*, 1999; Maxwell *et al.*, 1999)). Bioassays, using the WHO test kit, where twelve pyrethroids impregnated into polyester netting were tested against *A. gambiae* and *Aedes aegypti*, with 3 min exposure, showed that lambda-cyhalothrin was, after washing of nettings, one of the two treatments with the highest insecticidal activity (Lindsay *et al.*, 1991). Similar bioassays showed that lambda-cyhalothrin CS (10 mg/m²) treated bednets caused between 85 - 100% mortality in *A. gambiae* after 15 months and 50% two years post-treatment (Curtis *et al.*, 1996). With respect to sandflies, bioassays where wild *P. orientalis*, were exposed for 30 seconds to a netting cage impregnated with lambda-cyhalothrin (10 mg/ m²) showed 100% mortality of this sandfly species within 1 h (Elnaiem *et al.*, 1999b).

In contrast, in experimental huts, lambda-cyhalothrin has a very low insecticidal effect on *C. quinquefasciatus* (Curtis *et al.*, 1996; Maxwell *et al.*, 1999; Miller *et al.*, 1999). This differential action is explained by the high irritancy which lambda-cyhalothrin

causes on *C. quinquefasciatus* (Miller and Gibson 1994), which presumably prevents the mosquito to be in contact with the treated surface the necessary time for it to take a lethal dose. The high mortality of *L. longiflocosa* in the present study provides further evidence for the lack of excito-repellent effects of the lambda-cyhalothrin (which would have presumably reduced the mortality rate as for *C. quinquefasciatus*).

The relatively high mortality (48%) at 24 hours after exposure to the untreated bednet in the present study is within the expected range. Under field conditions a relatively high mortality at 24 h could be expected for some wild caught sandfly species because of difficulties in keeping adequate microclimatic conditions and due to damage during catching and handing of the sandflies. For instance the mortality of control groups, in relatively similar studies, were 22% for *L. youngi* (Alexander *et al.*, 1995c) and 43% for *L. spinicrassa* (Perruolo 1995). Taking this into account, the high immediate mortality (68%) observed in the insecticide treated bednet and the very low mortality in the control (5.6%) for this time, it is likely that the 24 h mortality in the ITN, due only to the insecticide activity, could be around 100%.

The immediate mortality (5.6%) observed in the untreated bednet was unexpected. As all the sandflies from this group were collected only during one night, from one house, it is suspected that this result could be due to a temporal contamination with insecticide. It could be possible that the householders used an insecticide (or other lethal substance) shortly before the test, in spite of the requests made by the research team not to use any insecticide during the study. The householder of this house had reported the periodic use of mosquito coils to control sandflies in the past (Chapter 5, section 5.3.2.1). Another possible explanation is that the contamination came from the ITN used the night before, but this is less likely because the residual effect of the insecticide would cause mortality for more than a single night. In conclusion, based on the low value and their isolated occurrence it is considered that the immediate mortality in the untreated bednet did not have a significant effect on the results.

Finally, the high immediate mortality observed inside the ITN (93%) was presumably due to the higher physical contact between sandflies and the insecticide which occurs when the sandflies cross the bednet.

4.4.2.4 Percentage of female flies resting temporally on the walls

A significantly lower percentage of *L. longiflocosa* females were found resting on the walls of the bedrooms with the ITN, compared with the bedrooms with the untreated bednet (2.6% vs 8.2%, respectively). The most likely explanation is not that sandflies in the treated room are more likely to escape due to an excito-repellency effect. Rather it is simply that a high percentage of sandflies collected in the treated room was knocked down. When knocked down sandflies are excluded from the analysis, no difference is observed in indoor resting behaviour: 7.7% (3 / 39) of females resting on the walls of rooms with the ITN versus 8.6% (24 / 278) resting on the walls with the untreated bednet ($z = -0.69, p = 0.493$).

4.4.3 Comparison of insecticide treated bednets and house spraying

This study was designed to compare the effectiveness of using ITNs and house spraying (both with lambda-cyhalothrin) under natural conditions on the probability of being bitten inside a house. The study was carried out 4 months after treatment, so that the results may reflect differences in the persistence effect of the two treatments. Hence, persistence was first measured by bioassays. The principal measure of effectiveness was the comparison of light trap catches in rooms where the villagers were sleeping as usual. Hence, it was also necessary to investigate the relationship between light trap catches and human landing rates in a subset of treated and untreated houses in order to interpret the light trap data correctly. It is important to note that the human landing rates measured in this study do not reflect the actual rates experienced by the villagers sleeping under the bednets, as the landing catches were made by researchers outside the bednets. In contrast, the human landing rates in the sprayed houses do provide a direct measure for comparing the risk experienced by the villagers in sprayed houses versus control houses.

4.4.3.1 Residual effect of the insecticide

The residual effect of nets and spraying 4 months post-treatment was measured by comparisons of immediate mortality, mortality 1 hour post-exposure, and 24 hours post exposure following a 3-minute exposure (for nets) or a 1-hour exposure (for sprayed walls). A significant reduction in 1-hour mortality rates was detected 4 months post-

treatment (compared with immediately post-treatment) for both nets and sprayed walls; there was also a significant reduction in immediate mortality rates and 24 hr mortality rates 4 months post-treatment on sprayed walls. Nevertheless mortality rates were still relatively high for both treatments after 4 months – with 24 hr mortality rates of 97% and 90% for nets and sprayed walls, respectively. However, it should be noted that average 24 hr mortality rates were also high in the controls: 47.4% for bednets and 55.6% for walls. These high percentages indicate that the recorded mortality in the treatments with insecticide included a strong effect of mortality for reasons other than insecticide activity, such as microclimatic conditions (temperature, humidity), manipulation and transport which could not be controlled. Effect of manipulation and transport was expected to be minimized by the practice of not using sandflies caught less than 12 h before the bioassays were carried out. Difficulties keeping wild-caught *L. longiflocosa* alive were also experienced during the efficacy study (section 4.3.1.3) where 24 h mortality of sandflies caught inside houses with untreated bednets was similar, 47.7%, to the mortality in the controls of the present study. Reported survival of controls in field bioassays is variable. For instance *L. longipalpis* presented very low 24 h mortality (from 0% to 13.8%) following exposure to unsprayed walls (Passerat De Silans *et al.*, 1998). In contrast, control bioassays with *L. verrucarum* on walls led to 24 h mortality rates from 8.2% to 52.8%, in bioassays (Davies *et al.*, 2000a). Taking into account the considerable mortality at 24 h caused by non-insecticidal factors, this discussion will focus mainly on the records of 1 h mortality, as 1 h mortality rates for controls were very low: 3.0% for walls, zero for bednets.

The high mortality of sandflies, 83.1% (mainly *L. longiflocosa* females) 1 h after exposure to 4 months treated and unwashed bednets made of polyester indicates that lambda-cyhalothrin CS, 25 mg/m², treated bednets kept their initial high insecticidal effectiveness at least for 4 months after treatment. This high 1 h mortality suggests that insecticide-induced 24 h mortality, the standard time established to measure insecticidal effect (WHO 1975), could be close to 100%. On the other hand, the significant reduction in 1 h mortality in the ITN after 4 months of treatment compared with the freshly treated bednet could indicate a reduction in effectiveness due to a quicker knockdown.

Literature regarding directly residual effect of insecticides on sandflies is scarce, especially for ITNs. However, as laboratory bioassays tend to suggest that sandflies are generally more susceptible to insecticides than mosquitoes (Oliveira and Melo 1994), it is likely that the many reported studies on the residual effect on ITNs for mosquitoes are relevant for sandflies. For example, an extensive review of studies on lambda-cyhalothrin CS (microencapsulate suspension) for treatment of mosquitoes nets concluded that this pyrethroid at a dose of 10 - 15 mg/m² remained effective for up to 11 months (WHO 2001) on unwashed treated bednets. Nevertheless, it should be pointed out that residual effects of bednets are influenced by the type of insecticide, formulation and type of fabric. For instance, field bioassays on *A. gambiae* in Tanzania using the WHO cones on bednets made of polyester and impregnated with lambda-cyhalothrin EC (emulsifiable concentration), 10 mg/m², showed that 24 h mortality was around 100% for fifteen months; while the mortality induced by bednets made of polyethylene and treated with the same insecticide, dose and formulation, dropped to 50%, five months after treatment (Curtis *et al.*, 1996). Another study which compared the residual effect of CS with EC formulations showed that CS formulation at five different doses (1 - 15 mg/m²) caused mortality rates of about 80% for at least 10 weeks, while EC was less effective, with mortality rates dropping in the first week at lower doses (Miller *et al.*, 1999). So the results of the present bioassay study on ITN causing almost 100% mortality 4 month after treatment are as expected.

The drop in effectiveness 4 months post-treatment for the sprayed houses appears to be more significant than that for the bednets, with reductions noted for all three mortality rates measured. This could explain, at least in part, the apparently lower effectiveness of spraying 4 months post-treatment detected in the trial. Although the reduction in mortality 4 months after spraying the walls was apparently not dramatic at 24 h post-exposure (90.1%), this result was relatively unexpected given previous reports of the apparently long residual effect of the lambda-cyhalothrin. Field bioassays with *L. verrucarum* in Perú, using the same insecticide and dose, but a wettable powder (WP) formulation, showed that on apparently similar surfaces (adobe walls) the initial effectiveness, 100% mortality, of lambda-cyhalothrin sprayed indoors was maintained for up to 6 months (Davies *et al.*, 2000a). In relation to other pyrethroids, bioassays carried out on *L. intermedia* in Brazil measuring the residual effect of deltamethrin, 25 mg/m², sprayed indoors on walls (presumably made of bricks) showed that 100% 24 h

mortality rates were maintained for at least 10 months after spraying (Falcão *et al.*, 1991). In contrast, other pyrethroids seem to have a lower residual effect. For instance, the 24 h mortality of *P. papatasi* exposure for 30 min to cement plastered wall sprayed with permethrin, 2000 mg/m², dropped to 86.2% at 1.5 months post-treatment (Morsy *et al.*, 1993). The reduction in the residual effect of lambda-cyhalothrin in the present study could be due to the features of the treated surface, as has been demonstrated in other studies. A recent study with *L. ovallesi*, in Venezuela, using the same insecticide, dose, but presumably different formulation than in the present study, showed that after two months treatment “bahareque wall” mortality dropped to 10% compared with 50% mortality on walls made of wood or cement (Felicangeli *et al.*, 2003).

4.4.3.2 Comparisons of female abundance in light trap catches

No significant differences in the measures of indoor sandfly abundance (catches by LT) were detected between treatments, with the exception that *L. nuneztovari* female abundance was 71% lower in the treated net rooms than in control houses. However, mean *L. longiflocosa* abundance was considerably lower in both treatments with insecticide compared with the control group. The reduction was apparently more evident for ITNs where the GM number of *L. longiflocosa* females was reduced by 48% (from 50 f/LT/n to 26 f/LT/n). Similar results were found by the study of Felicangeli *et al.* (2003) who found that indoor spraying with lambda-cyhalothrin reduce significantly the abundance of *L. ovallesi* caught by LT. Nevertheless, the effect last for only 1.5 months due probably to a quick reduction in the residual effect. It should be clear that the present study did not attempt to measure reduction in sandfly abundance at population level because the intervention was at household level, not at village level.

The lack of statistical significance in the reduction of *L. longiflocosa* indoor abundance with the catches by CDC light traps in both treatments with insecticide seems to imply that there was no effect of treatment on indoor female abundance. However, it is also possible that (1) the sample size was insufficient; or (2) the light trap catches did not provide an unbiased estimate of sandfly abundance in insecticide treated houses.

(1) Insufficient sample size. The number of replicates per treatment, 16 houses (48 houses in total) was lower than planned by the calculated sample size of 20 houses per

treatment (60 houses in total) to detect a 50% reduction in abundance, for 90% power at $p < 0.05$ (section 4.2.2.1). Hence, if treatment caused a reduction in indoor abundance of less than 50%, it would have been unlikely to detect it.

(2) Bias in the sample method. It could be possible that the sample method, CDC light traps catches (LTC), was affected by the insecticide treatments, and so provided an unreliable estimate of indoor abundance. Nevertheless, LTC correlated well with human landing catches (HLC); and – most importantly - no effect of the insecticide was detected on the effectiveness of LTC for assessing sandfly abundance, as indicated by the absence of any statistical difference in the mean log ratio, $\ln[\{LTC + 1\} / \{HLC + 1\}]$, between the control houses and either the houses with ITNs or the sprayed houses. This is consistent with an analogous study on indoor *L. verrucarum* catches in Perú where no effect of house spraying was found by comparing the log ratio of LTC and HLC in sprayed (lambda-cyhalothrin 25 mg/m²) and unsprayed houses (Davies *et al.*, 1995). Nevertheless, it should be pointed out that the effect of insecticide on the effectiveness of LTC could impact on other variables such as blood-fed sandflies, host preferences and seasonality (see below).

There is at least one previous report where treatment with insecticide affected sandfly sampling efficiency: in Alto Aguacatal, Colombia, where house spraying with deltamethrin, 500 mg/m², was evaluated by sticky traps (Alexander *et al.*, 1995a). Previous studies have shown that indoor sticky trap catches are generally well correlated with human landing rates in untreated houses (Montoya and Muñoz 1993). However, in Alto Aguacatal, sandfly abundance catches on sticky traps (*L. youngi*) in the treated houses was significantly higher compared with the control houses. This unexpected result could have two explanations: (i) after contacting the insecticide the sandflies were less mobile and spent more time in the house leading to a greater chance of coming into contact with the sticky traps (the hypothesis favoured by the authors of the study); or (ii) there was a highly irritant effect of the insecticide which lead the sandflies to rest more on the only non-irritant surface (the sticky trap). Whatever the explanation, it is clear that the efficiency of the sticky traps apparently increased as a result of house spraying, making it harder to detect any reduction in indoor abundance as a result of the treatment.

In this study, the lack of correlation between the log ratios, $[\ln\{\text{LTC} + 1\} - \ln\{\text{HLC} + 1\}]$ versus the combined measure of sandfly abundance $[\{\ln(\text{LTC} + 1) + \ln(\text{HLC} + 1)\} / 2]$ confirmed that, within the range of abundance observed, the relative sampling efficiency of the CDC traps was not density-dependent. This result is contrary to the study for *L. verrucarum* in Peru where the sampling efficiency of the CDC light traps was density-dependent. Because of the possible underestimation of the catches by human landing in the present study, the finding of no density-dependence of the CDC light traps should be interpreted with caution, not least because the human landing catches were only carried out during a part of the night (from 19:00 to 21:30), and not necessarily during the period of peak of sandfly indoors activity. Human landing rates, within the study area, during peak hours (21:30 - 00:30) rose to 15 s/p/3h in houses with high sandfly activity (section 4.2.1.1), compared to a mean of 5.8 f/p/2.5h for control houses from 19:00 to 21:30. The latter might also explain the relatively high ratio of LTC: HLC in this study compared to the *L. verrucarum* study. The overall GM ratio of CDC light trap catches to human landing was 7.4, which means that one CDC light trap (from 18:00 to 7:00) caught 7.4 times the number of *L. longiflocosa* females collected by a single human bait (from 19:00 to 21:30 h) in the same bedroom. This compares to an equivalent GM ratio of 3.2 calculated for *L. verrucarum* in Peru, where human landing catches were carried out throughout the night (Davies *et al.*, 1995).

4.4.3.3 Comparisons of blood-fed females in light trap catches

Both the number and percentage of blood-fed females caught by LTC in sprayed houses were significantly lower than in control houses. This appears to be contradictory to two findings: (1) the ratio of LTC:HLC was not affected by spraying (see section 4.3.3), and (2) the HLC in sprayed houses were almost the same as in control houses (5.9 f/p/2.5 h and 5.8 f/p/2.5 h, respectively) (see section 4.3.3). A similar combination of results was observed in an analogous study of *L. verrucarum* in Peru (Davies *et al.*, 1995), which detected a significant reduction (89%) in the percentage of blood-fed females in sprayed (lambda-cyhalothrin, 25 mg/m²) houses. In Peru, this reduction was explained by a reduction of effectiveness of LTC in catching blood-fed females. This is because fed sandflies tend to rest immediately after engorging, at least temporarily, so they would have a greater chance of being knocked down by the insecticide, and less chance of being caught in a CDC light trap. A similar phenomenon in this study could explain the

apparently lower GM ratio of LTC : HLC (5.4) in the sprayed houses compared with the GM ratio in the control houses (9.7), taking into account that fed females represented 20% of all caught females in control houses. These ratios indicate that while in the control houses a CDC light trap caught 9.7 times the number of *L. longiflocosa* females caught by a single human bait, in the sprayed houses a CDC light trap caught only 5.4 times the number caught by a human bait (i.e. because the LTC in sprayed houses fail to include most of the blood-fed flies). In conclusion, it is considered that a reduction in blood-fed sandflies in LTC after house spraying should always be interpreted cautiously, such as the reported reduction of more than 50% in the percentage of blood-fed females of *L. nuneztovari* females in indoor LTC after house spraying in Las Yungas with deltamethrin, 25 mg/m² (Le Pont *et al.*, 1989c).

A significant reduction in both the number (94.1%) and percentage (92.5%) of blood-fed females of *L. longiflocosa* collected by indoor LTC was also observed in houses with people sleeping under ITNs during the post-intervention trial. In this case, there is less reason to doubt that this implies a true reduction in human biting rates. Firstly, biases in the effectiveness of LTC for catching bloodfeds in rooms with ITNs are less likely as blood-fed sandflies resting temporarily on indoor walls will not come into contact with insecticide. Secondly, there is direct evidence from the efficacy study that sleeping under treated bednets reduces biting rates (section 4.3.1.2). The few bloodfeds collected by LTC during the trial in houses with nets are presumably from humans (or other hosts – see below) not under the bednets. Thirdly, while it is true that the LTC : HLC ratio in houses with nets was not significantly different from that in control houses, this result is not so relevant as in that study the human baits were outside the nets, not sleeping inside. Finally, further support comes from the direct evidence that HLC in houses with nets are generally lower than in control houses, even when the human baits are outside the nets (see section 4.3.3). To my knowledge this is the first report of a reduction in blood-fed sandflies as the result of sleeping under ITNs under natural conditions.

4.4.3.4 Comparisons of blood meal size amongst blood-fed females in light trap catches

Another remarkable finding was that the percentage of fed sandflies which were fully engorged was significantly lower in sprayed houses (60%) compared to control houses

(83%). This percentage was also relatively low in houses with nets (67%) but not significantly different from either of the other two sets of houses, not least because of the low sample size of bloodfeds in houses with nets ($n = 21$). These results provide evidence that the insecticide treatments reduce feeding success, possibly as the result of exposure to a sub-lethal dose. Taking into account that the amount of blood imbibed for sandflies is linked with fecundity (Ready 1979), it could be possible that this factor would contribute to a reduction in sandfly population in a large scale study. However, one cannot discount the possibility that in sprayed houses partially fed sandflies are more likely to be caught in a LTC than fully fed sandflies, if the former remain active after taking their partial meal in order to find a second meal, while the latter immediately search for a temporary resting site (i.e. the sprayed walls). It is also possible that partially fed sandflies tend to be sandflies that fed on non-human hosts on which they have lower feeding success. These could include outdoor hosts, if sandflies then enter the house to complete engorgement. In such circumstances a reduction in the proportion of meals taken on humans (see below) would also lead to an increase in the percentage of partially fed sandflies caught inside houses after insecticide treatment. The presence indoors of female sandflies which had fed outdoors has been reported previously for another exophilic species, *L. ovallesi* (Gómez *et al.*, 1998).

4.4.3.5 Comparisons of human blood index amongst blood-fed females in light trap catches

The HBI for all *L. longiflocosa* collected in the control houses was very high, 0.845, with the best estimate (for fully-blood females with fresh blood) of 0.951. This high HBI could be taken as an index of high anthropophagy. However, this value should be interpreted with caution as it probably does not reflect the feeding behaviour of the whole population of *L. longiflocosa*. This is because the LTC could be biased as that the catches were carried out in a site used exclusively for feeding, houses not being considered as diurnal resting sites for the exophilic *L. longiflocosa*. In addition this HBI is only representative of the high abundance season for sandflies, when the highest indoors biting activity takes place. The HBI may alter in other seasons where the ratio "vector : host" changes. For these reasons the value of the HBI for the control group should be considered as a guide and it is probably an overestimate of the actual HBI for *L. longiflocosa*. Confirmation of high anthropophagy of *L. longiflocosa* by the HBI

should include detailed study on feeding preferences, including sampling in resting sites outdoors. What is clear from the indoors HBI is that *L. longiflocosa* enters the houses to feed almost exclusively on humans.

There have been few previous measurements of indoor HBI for *Lutzomyia* species. In Chaute, Peru, the HBI for *L. verrucarum* and *L. peruensis* (endophilic vectors of CL) was calculated by the precipitin ring test in sandflies caught by LTC in three houses during one year. The results showed a HBI of 0.197 for *L. verrucarum* and of 0.403 for *L. peruensis* (Ogusuku *et al.*, 1994). Although these values are lower than the HBI recorded for *L. longiflocosa* in the present study indicating a possibly higher anthropophagy for *L. longiflocosa*, it is difficult to make a direct comparison of the indexes because the HBI of *L. verrucarum* and *L. peruensis* are probably less biased, as houses are thought to be used as resting sites for sandflies which may have fed outside. In El Ingenio, Venezuela (Gómez *et al.*, 1998), the HBI for *L. ovallesi* (apparently exophilic vector of CL) was calculated by dot-ELISA in sandflies caught with LTC in 29 houses, during 10 months. The result showed a HBI of 0.817 (using as denominator the number of sandflies whose blood content was identified) which is quite similar to the value found in this study. Finally, in El Callejón, Colombia, the HBI for *L. longipalpis* (endophilic vector of VL) was calculated by the precipitin test in sandflies collected resting in one house and in two sites outdoors for 16 months. The results showed a low HBI, 0.186, indoors and even lower HBI, 0.003 - 0.004, outdoors (Morrison *et al.*, 1993), indicating a very low anthropophagy. Whilst the results of these four studies are not fully comparable, it appears that *L. longiflocosa* is amongst the *Lutzomyia* species with relatively high endophagy and anthropophagy.

The significant proportional reduction of 68% in the HBI in the houses with ITNs is principally because of the proven reduction in bloodmeals taken on protected humans. However, some of the effect could feasibly be as the result of sandfly diversion from humans to other hosts, probably dogs, chickens and pigs around the houses. This assumes that *L. longiflocosa* is an opportunistic species, behaviour which has been shown for other vectors of leishmaniasis, such as *L. spinicrassa* (Alexander *et al.*, 1992), *L. youngi* (Añez *et al.*, 1994), *L. trapidoi* (Tesh *et al.*, 1971), *L. verrucarum* and *L. peruensis* (Ogusuku *et al.*, 1994) and *L. longipalpis* (Morrison *et al.*, 1993). Diversion could occur as a result of excito-repellent effect of the lambda-cyhalothrin, or

simply because failure to find an exposed host inside a house stimulates sandflies to leave and find alternative blood sources. This seems unlikely as (1) no evidence of excito-repellent effects was detected in the efficacy study (section 4.4.2.1); and (2) no evidence of any increase in bloodmeals taken from non-human hosts was detected as the result of providing treated nets. Prior to the intervention, the total number of bloodfeds collected by LTC in the 16 “net houses” was 229 (Table 4.10), of which it is possible to assume 95.1% were taken from humans (Table 4.13) leaving 11 bloodfeds on non-human hosts. After the intervention, 21 bloodfeds were collected in the same houses (Table 4.11) of which 37.5% can be assumed were taken from humans (Table 4.13), leaving 13 on non-human hosts (i.e. no difference). During the same time period the number of bloodfeds in the control houses was also stable (from 558 to 499). Clearly, confirmation of diversion would need additional studies involving comparison of blood contents in daytime resting sites.

The observed HBI was also significantly reduced in sprayed houses. For the reasons described above, the possibility can not be discounted that sandflies feeding on humans inside houses are less likely to be caught in light traps as the result of house spraying. In contrast, sandflies that fed outside presumably entered the house not to rest but because they were attracted by the light trap, and so may not have been exposed to the sprayed, inside walls. Using the same calculations as above, the estimated number of sandflies with bloodmeals from non-human hosts collected in indoor LTCs actually increased from 14 (in the 16 “sprayed houses” pre-intervention) to 42 after the same houses had been sprayed. Whether this suggestion of diversion is real requires much further evidence.

The results of the present study showed that there was a significant association between both blood condition (a measure of blood digestion) and bloodmeal size on the identification of the blood meals – presumably by their effects on the sensitivity of the precipitin test to detect the antigens. So, human blood which came from females with fresh and full blood had the highest chance to be identified – leading to our best estimate of 0.951 for the HBI of *L. longiflocosa* in untreated houses (Table 4.13). The effect of blood condition and amount on blood identification was previously related with the identification of blood meals in *L. ovallesi* in El Ingenio, Venezuela (Gómez *et al.*, 1998). The lower HBI in that study, 0.403 (when all tested sandflies were included

in the denominator), compared with the HBI in the present study for the control houses, 0.845, could be explained, at least in part, by the fact that in the present study only females with a relatively high amount of blood (at least half of the abdomen with blood) were tested, while in the study of Gómez *et al.* females with any blood amount were included; this reduced the chance of identification of human blood in the samples.

Considering blood condition, it has been shown that using the precipitin test reliable identification of blood meals can be made in sandflies (*L. trapidoi*) up to 18 h after feeding (Tesh *et al.*, 1971). Taking into account that most of the sandflies sampled during the present study were fed less than 12 h previously (the working time of each CDC light trap) it appears that most of the blood-fed females tested in the present study were in an appropriate condition for the test.

The possibility, mentioned earlier, that partially fed sandflies may include a relatively high proportion of sandflies that had fed outside and had come into the house to complete engorgement would provide an alternative explanation for the relatively low percentage of partially feds with a positive human blood identification. However, this is a less parsimonious explanation than that provided by the impact of bloodmeal size on sensitivity (already demonstrated in pilot studies using sandflies fed on known hosts), and there is no supporting evidence. If partial feds were biased towards sandflies recently entering the house after having just taken a small bloodmeal outside, we might expect them to have a higher percentage of fresh blood than fully engorged sandflies. But, in fact the percentage with fresh blood of half-fed sandflies was 43% (25 / 58) compared to 46% (216 / 467) for fully engorged sandflies. It can, therefore, be concluded that there is no reason to believe that the percentage of human bloodmeals taken by partially fed sandflies is any different from that taken by fully-fed flies.

5 KNOWLEDGE, ATTITUDES AND PRACTICE ON SANDBLIES AND CUTANEOUS LEISHMANIASIS CONTROL

5.1 INTRODUCTION

5.1.1 Overview on knowledge, attitudes and practice on leishmaniasis

The knowledge, attitudes, and practice (KAP) of a population with respect to a given insect vector-borne disease are important factors to take into account in order to implement any control programme and guarantee its sustainability. They also can provide a baseline against which one can measure, in part, the impact of a control programme.

Relatively few studies of KAP on leishmaniasis, whether cutaneous leishmaniasis (CL) or visceral leishmaniasis (VL), have been published to date. Table 5.1 summarises the most recent studies, most of them in Latin America. Their methods have been, mainly, quantitative (although some included a qualitative part) by administration of questionnaires to one or all the adult members of households, asking mainly about the knowledge and practice related to the disease, the vectors and the control of the disease. There is a wide variation in knowledge and practice in the listed studies which could be due to one or more of the following factors: differences between cultures, ethnic groups, socio-economic status, isolation of the study areas, cover and quality of health services, degree of human-vector contact, and sites of transmission. Nevertheless, some general conclusions can be drawn: 1) in most of studies (7 / 8) there was a relatively high (> 75%) knowledge of the diseases with the exception of the study in Nepal on VL, where the knowledge of the disease was lower (< 50%) (Koirala *et al.*, 1998); 2) only three studies have investigated the knowledge of sandfly vectors (Weigel *et al.*, 1994; Alves *et al.*, 1998; Arana *et al.*, 2000). This knowledge was highly variable, from < 25% to 98%; 3) knowledge of sandflies' role in transmission seems lower than that on disease or sandflies and had an apparent large variation, from < 10% to 60%. The higher

Table 5.1 Some recent studies in relation to knowledge, attitudes and practices about leishmaniasis.

Disease type	Country and locality (Reference)	No. interviewees (No. households or houses)	Leishmaniasis			Sandflies		
			% knew	% knew treatment ^d	% knew control	% knew	% knew role in transmission	% knew control
CL	Brazil, Bahia (Santos <i>et al.</i> , 2000)	851 (168)	NA	NA	NA	NA	NA	43 ^b
	Colombia, Valle del Cauca (Vásquez <i>et al.</i> , 1991)	332, representing 2092 inhabitants, which were approximately 20% of total population	80	0	28 ^e	NA	NA	NA
	Colombia, Chocó (Isaza <i>et al.</i> , 1999)	345 from a population of 1553, >14 years old	94	<10	<25	NA	35 ^b	NA
	Costa Rica, Cantón de Acosta (Dobles-Ulloa & Perriard, 1994)	48 in 12 houses (170)	>75 ^a	NA	0	NA	>75 ^a	0
	Ecuador, Pichincha (Weigel <i>et al.</i> , 1994)	208 from a total population of 3985 inhabitants	>75 ^a	7	NA	98	<10	NA
	Guatemala, El Petén (Arana <i>et al.</i> , 2000)	425 (1097)	97	50	15	<25 ^b	60 ^b	NA
VL	Brazil, Maranhao (Alves, <i>et al.</i> , 1998)	283	94	6	22	49 - 80 ^b	20 - 51 ^b	NA
	Nepal, Morang (Koirala <i>et al.</i> , 1998)	Heads of households from two villages with total population of 2197 inhabitants	<50 ^a	NA	1 - 2	NA	<25 ^a	0
	Border Uganda / Kenya, Pokot (Chappuis & Cavailler, 2002)	292 households representing a population of 1929 inhabitants	95	NA	22 ^a	NA	61 ^f	NA

NA: Was not evaluated or the information was not given; ^a Approximate percentage assigned based on the reports of: low or few (< 25%), less than half (< 50%), or most (> 75%); ^b Indirect evidence for reference to mosquitoes, insects or arthropods in general; ^c Indirect evidence as inhabitants referred that to avoid contact with the forest prevents the disease; ^d Refers only to use of antileishmania medicaments; ^e Refers to use of bednets only; ^f Include people who belief that VL could be contracted in other ways.

percentage of knowledge on sandfly role (> 75%), recorded in the study of Dobles-Ulloa and Perriard (1994), was not taken into account because the conclusions of this study may be disputed due to inconsistencies in the data collected and its small sample size; 4) regarding treatment, it seems that the current medicaments are not well known, with knowledge ranging from 0 to 50%, in all the studies (five) which considered this subject. Use of traditional treatments were common in most of the studies (Weigel *et*

al., 1994; Dobles-Ulloa and Perriard 1994; Isaza *et al.*, 1999); 5) knowledge on disease control was low in general, ranging from 0 to 28%; and 6) knowledge on sandfly control was investigated in only three studies and was also relatively low, from 0 to 43%.

Two additional unpublished studies should be mentioned. The first was a study on VL in Iran. This study was conducted in parallel with an intervention with insecticide impregnated dog collars, where 18 villages were sampled and around 1800 questionnaires were answered. It was found that only 45% of the responders knew the disease; 68% recognized the sandflies; and only 10% believed the disease was transmitted by sandflies (<http://www.emro.who.int/tdr/frs/proj00-80-edited.pdf>). These results are in concordance with the studies mentioned previously.

The second study was carried out in the Sub-Andean region of Colombia by Nicholls *et al.* (1998) in three endemic areas of CL. This study included part of the study area (La Troja and other villages of Tello municipality in Huila department) of the present thesis. A questionnaire was answered by 398 householders, most (281) of them from Huila department. In Huila most of householders, 83%, knew the disease; more than 80% of householders recognized the sandflies; more than 70% of householders seemed to recognize the sandfly role in disease transmission; apparently most of householders knew the appropriate medical treatment; and only 48% of them knew any type of disease control. Knowledge of sandflies and their role in transmission were both apparently remarkably high. The high knowledge of sandflies could be explained by the high human-vector contact indoors within the study area (see Chapter 3, section 3; and Chapter 4, section 4.3.2.3); while the apparently high knowledge of the role in transmission could be the result of a misleading question as explained below (section 5.1.2).

Finally, an important aspect in the practice of control measures at the household level is whether they are affordable. At the moment, the only published study on this subject is that of Santos *et al.* (2000). They investigated the association between family incomes and the use of control measures against arthropods in a CL endemic area near Bahia, Brazil (Table 5.1). They found that households with lower incomes (less than three minimum Brazilian salaries) practised fewer, 2%, control measures, which demanded an expenditure, compared with the control, 15%, practised by households with higher

incomes (more than three minimum salaries). However, no significant difference was found, probably due to the very low percentage of households who used measures (4.2%). The restrictive effect of family incomes in control was reinforced by the fact that households with the lowest incomes (less than 1 minimum Brazilian salary) did not use any costly control measure (bednets, mosquito coils, and repellents).

5.1.2 Outline and Rationale

It is remarkable that all but one of the cited KAP studies (Santos *et al.*, 2000) only asked householders about activities designed to control disease, and failed to address measures aimed at sandfly control per se. Given the poor knowledge of the role of sandflies in transmission, this is a serious omission. Control activities may be motivated by the nuisance caused by bloodsucking sandflies. Hence, this study was designed to investigate both CL and sandfly control activities. Control activities are likely to be determined not only by the perception of the problem (caused by either sandflies or disease), but also by economics – i.e. affordability will depend on income. Hence, this study also addresses the relationship between economic status and control activities. While the previous KAP study in the region (Nicholls *et al.*, 1998) provided much useful information, it was not designed to address either sandfly control per se or the impact of economic status. It also failed to provide some key data on CL control activity (e.g. frequency of use). Finally, its conclusions on knowledge of the role of sandflies as vectors of CL need to be interpreted cautiously as the questionnaire incorporated leading questions, i.e. "Which of these insects (a bug or a sandfly) could cause CL?".

The study described here involves a questionnaire applied during the House Risk Factor study (Chapter 3). The study is divided into three main sections. The first section concerns the general knowledge of CL, sandflies and control. This section also explores the relationship between knowledge of sandfly role in transmission and practice of control. Finally, a qualitative evaluation of the householders general knowledge (integral understanding) was carried out based on the presence/absence of each category of knowledge (CL, sandflies, role in transmission, and CL control). The second section addresses control measures. Here the four main control measures (smoke, bednets, house spraying with insecticides, and house spraying with non-insecticidal substances)

are described as well as their frequency of use. The third section describes the practice of control measures in relation to economic status using a qualitative index, based on some house features (wall type, wall cracks, presence of ceiling, total openings, presence of electricity) and the presumed ownership of domestic animals (pigs, equines, and cows).

5.1.3 Objectives

The overall aim is to describe the knowledge of CL and sandflies amongst households in the epidemic area of Huila department, to describe the control measures they use, and address what factors may determine household variability in these control activities. The specific objectives are:

1. To determine the level of the inhabitants' knowledge of CL and sandfly vectors.
2. To determine the inhabitants' knowledge of the role of sandflies in the transmission of CL.
3. To investigate the relationship between knowledge of the sandfly role in CL transmission and the practice of control measures.
4. To describe in detail the household control measures used against CL and/or sandflies.
5. To investigate the relationship between economic status of households with the practice of the control measures.

5.2 METHODS

Evaluation of the knowledge and practice of human populations in relation to CL and vector control of sandflies was carried out in a section of the main questionnaire of the cross sectional study, and answered by the heads of households, during the House Risk Factor study (Chapter 3), in La Troja, Brasilia, and El Cedral villages. A detailed description of the survey methodology is given in section 3.3 of Chapter 3. Only households who had lived for at least one year, in each of the sampled houses, were included in the study because they are more likely to know the vector. The vocabulary

used in the questionnaire was chosen based on the experience gained in the study carried out by Nicholls *et al.* (1998) with some modifications. To identify the parallel use of control measures for disease, by those householders who did not recognize the role of the sandflies in disease transmission, but did control against them, the same set of questions used about CL control was used again in a second section of the questionnaire, this time referring to the control measures applied against sandflies. Questions about identification of disease and sandflies by householders were not studied in depth, as the previous study showed that most of the population knew them very well. Information in relation to the different control measures included: date of start and frequency of use (i.e. all the time, only during the sandfly season [as specified by the interviewee] or other, as specified). Previous sampling of sandflies (unpublished data, Laboratorio de Entomología, INS) suggested sandflies are at higher abundance during the dry seasons, which occur twice a year. When the interviewee reported the use of spraying or smoke, information on the product name, or material used as fuel, was recorded. If the interviewee reported the use of bednets, the interviewer then measured the mesh size, as mesh size is likely to impact on the effectiveness of the bednets (ITN had not previously been used in the study villages with the exception of El Cedral where some ITNs were delivered in 1999). Bednets were classified into two categories: wide mesh size (≥ 1 mm) and narrow mesh size (< 1 mm). Only the control measures applied by the inhabitants in the current house were recorded. Finally, the date of any eventual intervention with spraying by the Health Service of the respective village (see Annexe 3.1, sections IV and V) was recorded.

The evaluation of economic status was indirect and based on the categorization of various house features. Building features chosen as indicators of a "better" economic status were: walls made out of bricks, few or no cracks in walls (0 - 30%), presence of a ceiling, few or no openings in the house (0 - 5.8 m²), and availability of electricity service; while the indicators of "low" economic status were: walls made out of "bahareque" or other material, many cracks in walls ($> 30\%$), absence of a ceiling, many openings in the house (> 5.8 m²), and no electricity service. Ownership of pigs, cows, and equines was considered as an indicator of "better" economic status. An index of economic status was obtained by summing the values of all eight features considered as indicators of economic status (presence = 1, absence = 0), so the index ranged

between "0" for the lowest status and "8" for the highest. Index values were then compared with the frequency of practice of each of the main control measures.

Statistical analysis was carried out by comparing the frequencies of knowledge of the disease, sandflies, and control measures using the χ^2 test with Yates' correction, and Fisher's exact test, as appropriate. Univariate regression analysis (using GLIM, with the assumption of normal distribution) was performed to test for an association between indoor sandfly abundance (log transformed data of *L. longiflocosa* females/LT/night) and the practice of any kind of control measure.

5.3 RESULTS

A total of 85% (249 / 293) of householders, who were interviewed in 271 of the houses during the risk factor study (Chapter 3), were included in the study, as they had lived in the house for at least 1 year. Four additional householders were recorded as not responding because the families were absent during the sampling.

Table 5.2 shows the composition of the interviewed householders by age and gender and total population sampled. Most of the interviewed householders were females (58.2%). By age, most (84.7%) of the householders were adults between 18 to 60 years of age. Regarding the total population (1244 inhabitants), an inverse pattern, by gender, was obtained compared with the householders: males constituted 55.5% of the total population.

5.3.1 General knowledge of CL, sandflies and control

5.3.1.1 Knowledge of CL, sandflies and their role as vectors

Figure 5.1 shows the different combinations of knowledge on CL, sandflies and the role of the sandflies in the transmission of CL, as well as for control measures, according to the answers of the householders.

A high percentage, 85.9% (214 / 249), of the interviewed householders, who had lived in the three study villages for at least one year, knew CL (which is known as leishmaniasis). By village, the same pattern was observed, with no significant

Table 5.2 Description of the interviewees and total population sampled that were included in the KAP study.

		Village							
		La Troja		Brasilia		El Cedral		Total	
		n	(%)	n	(%)	n	(%)	n	(%)
Total houses		81		89		67		237	
Number of families per house									
	1	80	98.8	83	93.3	64	95.5	227	95.8
	2	1	1.2	5	5.6	2	3.0	8	3.4
	3	0		1	1.1	1	1.5	2	0.8
Interviewed householders									
Gender									
	Females	41	50.0	58	60.4	46	64.8	145	58.2
	Males	41	50.0	38	39.6	25	35.2	104	41.8
	Total	82		96		71		249	
Age groups									
	18 - 60	61	74.4	90	93.8	60	84.5	211	84.7
	> 60	21	25.6	6	6.3	11	15.5	38	15.3
	Total	82		96		71		249	
Population									
Gender									
	Females	178	44.2	232	45.1	143	43.7	553	44.5
	Males	225	55.8	282	54.9	184	56.3	691	55.5
	Total	403		514		327		1244	
Age groups ^a									
	<18	166	41.3	253	49.5	126	38.7	545	44.0
	18 - 60	193	48.0	236	46.2	171	52.4	600	48.4
	> 60	43	10.7	22	4.3	29	8.9	94	7.6
	Total	402		511		326		1239	

^a Five missing data.

heterogeneity ($X^2_{(2)} = 5.56, p = 0.062$) (Figure 5.2). Nevertheless, the percentage of knowledge of CL in Brasilia village, 91.7% (88 / 96), was statistically significantly higher than in El Cedral, 78.9% (56 / 71), which was the lowest ($X^2 = 4.60, p = 0.032$). No significant differences were found for gender or age.

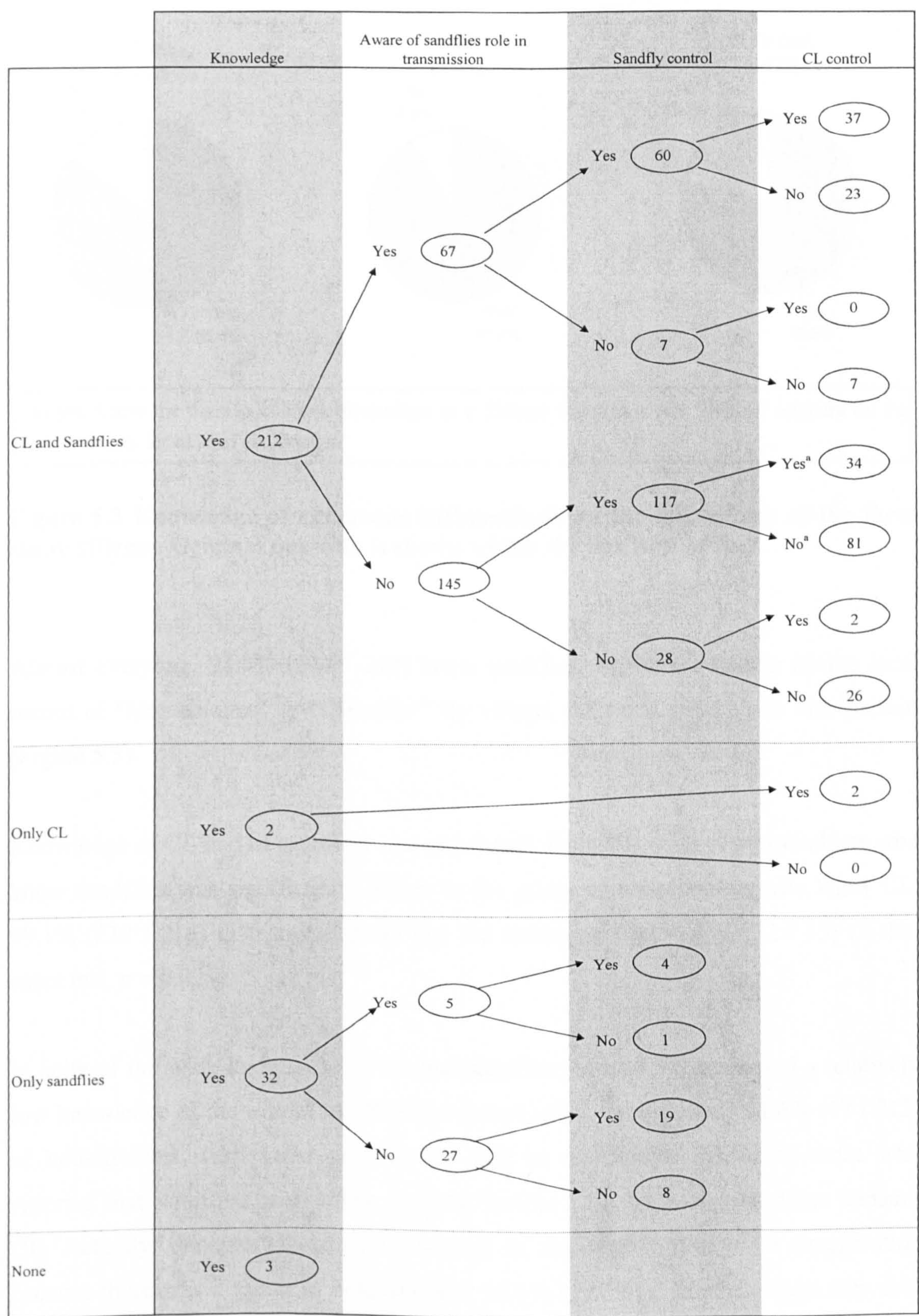


Figure 5.1 Summary of the knowledge and control of cutaneous leishmaniasis (CL) and sandflies by householders, who lived at least 1 year in the sampled house (n = 249). All possible combinations are obtained by the intercepts between columns and rows. ^a Two missing data in any of the two possible answers.

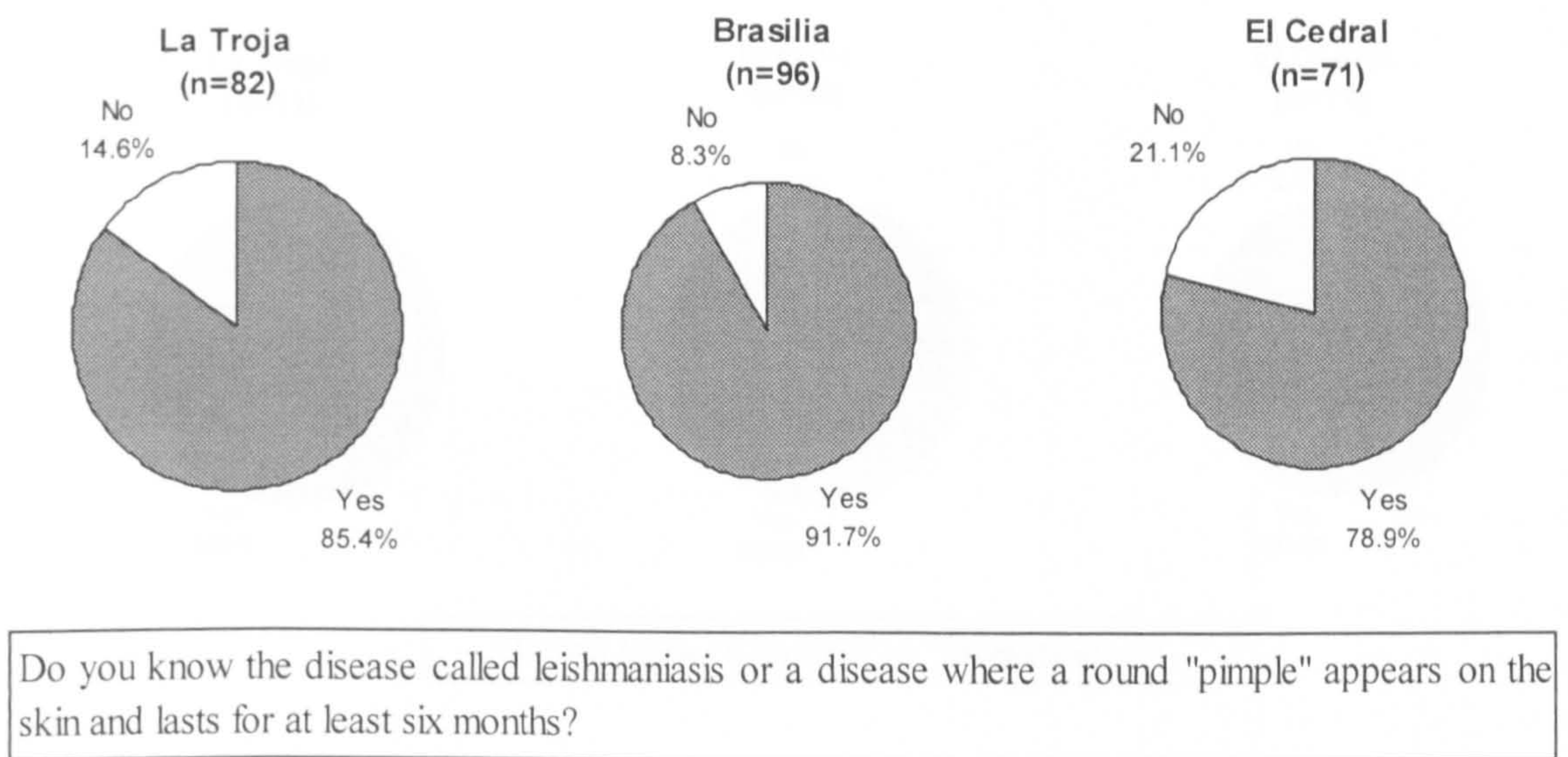


Figure 5.2 Knowledge of cutaneous leishmaniasis by the inhabitants of the three study villages. Original question is shown within the box (n = 249).

Almost everyone, 98.0% (244 / 249) knew sandflies, which are known by the local names of "Mantablanca" or "Capotillo". By village, the same pattern was also present (Figure 5.3).

Knowledge of CL and of sandflies was associated. The proportion of householders who knew sandflies was significantly higher in the group of householders who knew CL, 99.1% (212 / 214) than in the group that did not know CL, 91.4%, (32 / 35) (Fisher exact test, $p = 0.021$).

In spite of the wide knowledge of CL and sandflies, householders showed a relatively low knowledge of the role of sandflies as vectors of CL. In total only 31.6% (67 / 212) of householders, who knew sandflies as well as the disease (including those who reported that sandflies cause other diseases, besides CL), knew that sandflies transmit CL. Actually, this percentage of knowledge of the sandfly role is an exaggeration because there was a group of householders, 16.4% (11 / 67), who, although they said they knew that sandflies transmitted CL, also added that they did not believe it. Some of these people supported their point of view referring to their own experience, e.g. saying:

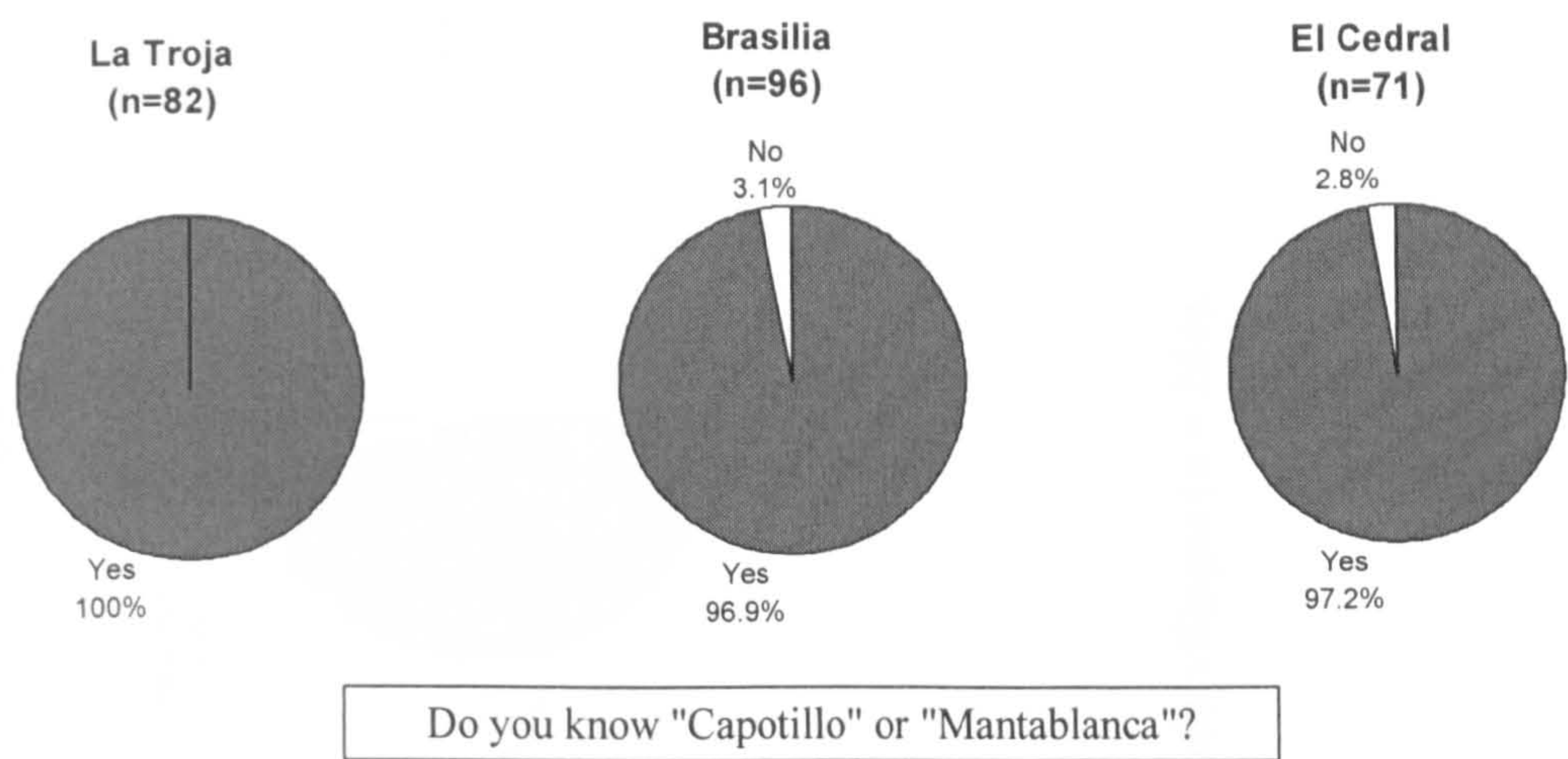
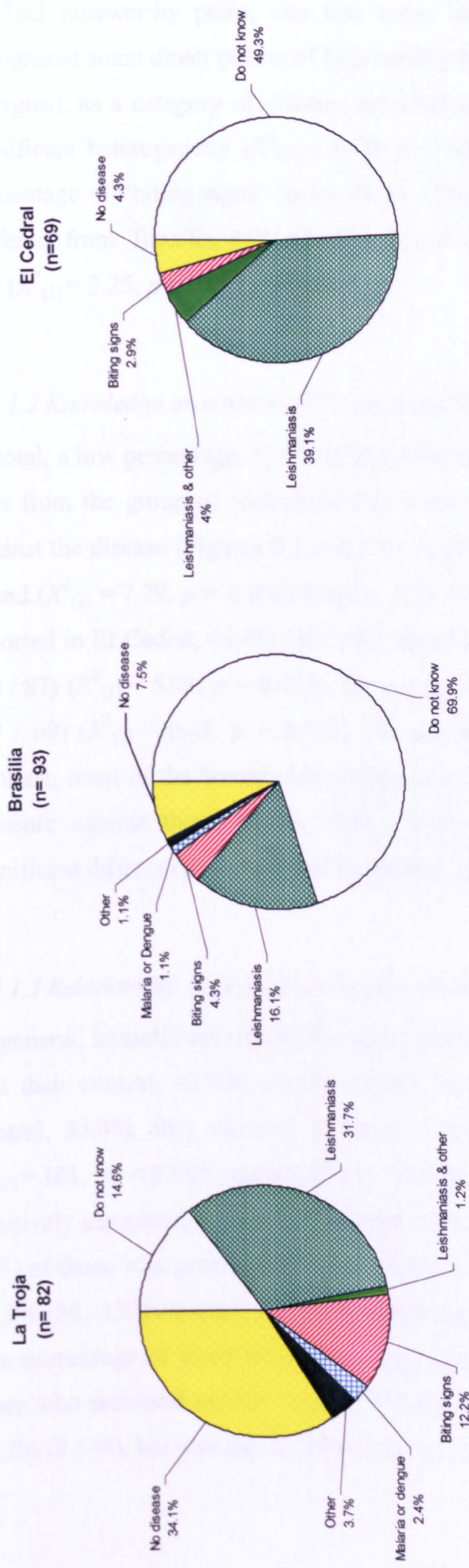


Figure 5.3 Knowledge of sandflies by the inhabitants of the three study villages (n = 249).

"Mantablanca do not transmit CL because we have been bitten by a lot of them and we have not got CL". Excluding this group, the percentage of householders who knew the sandfly role decreases to 26.4% (56 / 212). Surprisingly, a small group of householders, from the group who knew only the sandflies (5 / 32), also said they knew that these insects transmitted CL, although they did not know the disease (Figure 5.1). By village, the knowledge of the sandfly role in the transmission of CL showed significant heterogeneity ($X^2_{(2)} = 14.94, p < 0.001$) (Figure 5.4), a significantly higher knowledge was shown in El Cedral, 43.5% (30 / 69); compared with Brasilia, 16.1% (15 / 93) ($X^2 = 13.44, p = 0.001$), but, not for La Troja, 32.9% (27 / 82) ($X^2 = 1.35, p = 0.244$). No statistical differences were found by gender or age.

It is also important to note the high percentage of householders who are unaware of any role of the sandfly as a vector of any disease, or who did not perceive it as a nuisance (those who responded: "No disease" or "Do not know") which accounted in total for 61.1% (149 / 244). By village, there was a significant heterogeneity ($X^2_{(2)} = 17.27, p < 0.001$), the highest percentage of householder unawareness was in Brasilia, 77.4% (72 / 93). This percentage was significantly higher compared with La Troja, 48.8% (40 / 82) ($X^2 = 14.29, p < 0.001$), and El Cedral, 53.6% (37 / 69) ($X^2 = 9.14, p = 0.002$) (Figure 5.4).



Which disease is caused by "Capotillo" or "Mantablanca"?

Figure 5.4 Knowledge of the role of the sandflies as vectors of CL by the householders of the three study villages (n = 244).

A final noteworthy point, was that some householders, 6.6% (16 / 244) in total, recognized some direct effects of high sandfly biting (i.e. weal, ampulla, itch, fever, and allergies), as a category of disease, recorded as "biting signs". By village there was a significant heterogeneity ($X^2_{(2)} = 6.58, p = 0.010$). Nevertheless, the apparent higher percentage of "biting signs" in La Troja village, 12.2% (10 / 82) was not statistically different from Brasilia, 4.3% (4 / 93) ($X^2_{(1)} = 2.69, p = 0.101$), or El Cedral, 2.9% (2 / 69) ($X^2_{(1)} = 3.25, p = 0.071$) (Figure 5.4).

5.3.1.2 Knowledge on control of CL and sandflies

In total, a low percentage, 35.4% of householders who knew CL (75 / 212, two missing data from the group of control for CL were excluded) practised any control measure against the disease (Figures 5.1 and 5.5). Significant differences between villages were found ($X^2_{(2)} = 7.29, p = 0.026$) (Figure 5.5). The highest percentage of CL control was reported in El Cedral, 46.4% (26 / 56), significantly higher than that in Brasilia, 25.3% (22 / 87) ($X^2_{(1)} = 5.91, p = 0.015$), but not significantly different from La Troja, 39.1% (27 / 69) ($X^2_{(1)} = 0.41, p = 0.523$). No differences were found by gender or age. In contrast, most of the householders who knew sandflies practised some kind of control measure against them, 82.0% (200 / 244) (Figure 5.1 and 5.6). No statistically significant differences were found by village, gender or age.

5.3.1.3 Relationship between knowledge and the practice of control measures

In general, householders showed a significantly higher knowledge of sandflies, 98.0%, and their control, 82.0%, compared with the corresponding knowledge, 85.9%, and control, 35.4%, they claimed to practise against CL, ($X^2_{(1)} = 22.9, p < 0.001$; and $X^2_{(1)} = 101, p < 0.001$, respectively). The practice of CL control was significantly positively associated with the knowledge of the sandfly role in transmission: 50.7% (37 / 73) of those who practised CL control knew the sandfly vector role compared to only 21.9% (30 / 137) amongst those who did not practise CL control ($X^2 = 16.9, p < 0.001$). The percentage of those who knew the sandfly vector role was also higher amongst those who practised sandfly control, 32% (64 / 200), than amongst those who did not, 18.2% (8 / 44), but not significantly ($X^2_{(1)} = 2.68, p = 0.102$).

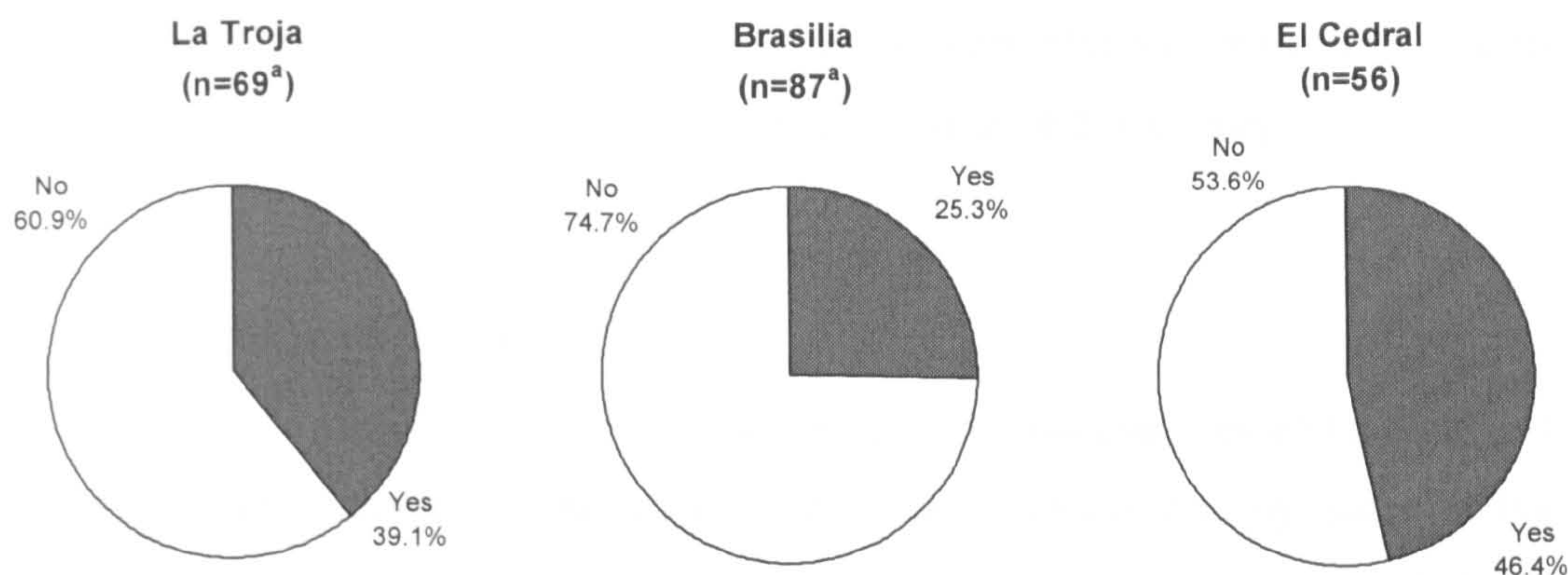


Figure 5.5 Percentage of householders who believed they practised any kind of control for cutaneous leishmaniasis (n = 212). ^aOne missing data point not included.

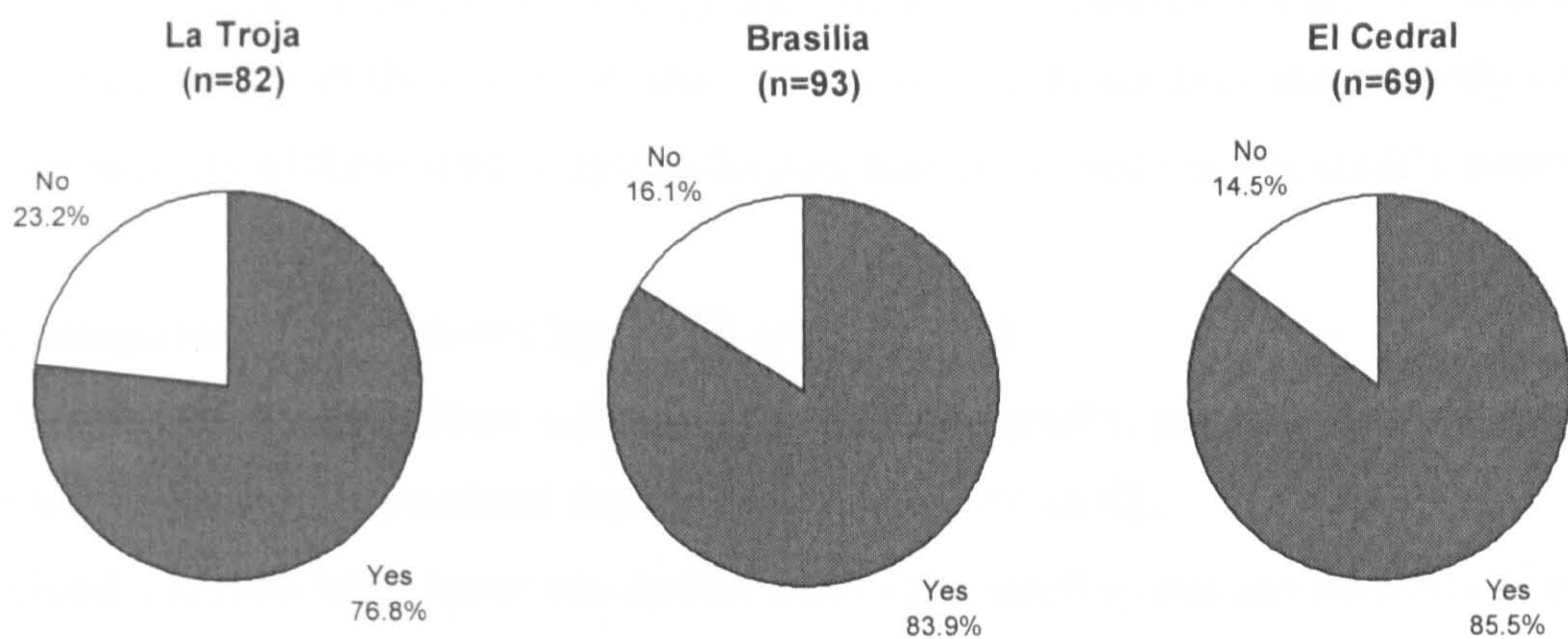


Figure 5.6 Percentage of householders who practised some kind of control measure for sandflies (n = 244).

Validation of the knowledge of the role of sandflies in transmission was obtained by comparing practice of CL control and sandfly control within the group of householders who knew the role of the sandflies in the transmission of CL. It was expected that if the householders who knew the vectorial role of sandflies practise any control for sandflies they should also report that they practise control of CL. Indeed, 75.7% (28 / 37) of householders who knew the role of sandflies reported the same control measures for sandflies and CL. Conversely, if the householders who knew the sandfly vector role reported no control measures for sandflies, it was expected that they should give the

same answer for the control of CL. In this case, the agreement was 100% (7 / 7). Hence, these findings imply that the majority of householders who said they knew the role of sandflies in CL transmission had a good understanding of this concept.

5.3.1.4 “Integral understanding” of CL

A general evaluation of the total knowledge or "integral understanding" of the knowledge and practice of the householders was carried out by categorizing the knowledge based on the presence or not of four main components: a) knowledge of the disease, b) knowledge of the sandflies, c) knowledge of the role of sandflies in the transmission, and d) control of the disease; where the presence of all of these was considered as the best knowledge. Practices of control for sandflies were not included because, although they could have an effect on the incidence of the disease, they do not have an effect per se on the knowledge and practice of householders on CL. In addition, the comparison with the results of other studies will be easier excluding sandfly control, as the majority of these studies had addressed disease control but not sandfly control.

Six categories of total knowledge on CL were defined:

- 1) Very good. Householders knew the disease, the sandfly, the role of the sandflies in the transmission, and practised any measure of control for CL.
- 2) Good. Householders knew the disease and/or the sandfly, and any measure of control for CL.
- 3) Acceptable. Householders knew the disease, the sandfly, and the role of the sandflies in the transmission of the disease.
- 4) Bad. Householders knew the disease and sandflies, but were unaware of any association between them.
- 5) Poor. Householders knew only the sandflies. Although, some also recognized the role of sandflies in transmission of disease, they did not know how the disease is transmitted.
- 6) None. No knowledge of sandflies or sandfly-borne diseases.

Table 5.3 shows the integral understanding of CL by householders. Most householders had a "bad" or "poor" understanding of CL, 57.5% (142 / 247). "Very good"

Table 5.3 Integral understanding on CL by the householders, accordingly to their knowledge on disease, sandflies, and the practice of any control of the disease.

Level of understanding	Knowledge on			Practice control for CL	n	% ^b
	CL	Sandflies	Role of sandflies			
Very good	x	x	x	x	37	15.0
Good	x	(x)		x	38 ^a	15.4
Acceptable	x	x	x		30	12.1
Bad	x	x			107 ^a	43.3
Poor		x	(x)		32	13.0
None					3	1.2

^a One missing data point in practice of control for CL was not included; ^b Denominator was 247; (): Indicates that the knowledge was or was not present.

understanding of CL was present in 15.0% (37 / 247) of householders. In total only 30.4% (75 / 247) of householders believed they practised some measure of control for CL. It is important to note that most householders, 77.1% (81 / 107), within the group of householders classified as having "bad" understanding of CL, practised some control of sandflies.

5.3.1.5 Relationship between sandfly abundance and control of sandflies

The geometric mean (GM) indoor abundance of *L. longiflocosa* females was 2.7 times higher in houses (n = 194) where the householders reported some kind of control of sandflies, 6.5 (5.1 – 8.2) s/LT/n, than in houses (n = 43) where the householders did not practise any control measure against these insects, 2.4 (1.3 – 3.9) s/LT/n. This difference was statistically significant ($F_{(6, 235)} = 2.75, p = 0.013$).

5.3.2 Control measures

5.3.2.1 Main types of sandfly control measures applied by householders

In general, the control measures reported to control CL were also reported for the control of sandflies, 62% (44 / 71). Taking this into account, and the fact that the practice of control measures against sandflies was dominant, compared with the practice of CL control (82% and 35.4%, respectively), this section will give the results for sandfly control only.

Four control measures were reported as the most practised by householders who knew sandflies: 1) smoke, 2) bednets, 3) house spraying with insecticide, and 4) house spraying with non-insecticidal substances. Most householders practised either one, 49.5% (99 / 200), or two, 39.0% (78 / 200), control measures. The remaining householders, 11.5% (23 / 200), practised three or more control measures against sandflies.

Table 5.4 shows the control measures practised by householders against sandflies. Smoke was practised by 62% (124 / 200) of the householders, significantly more than bednets, 36.5% (73 / 200) ($\chi^2_{(1)} = 25.01, p < 0.001$), house spraying, 33.0% (66 / 200) ($\chi^2_{(1)} = 32.57, p < 0.001$), or house spraying with non-insecticidal substances, 23.0% (46 / 200) ($\chi^2_{(1)} = 60.65, p < 0.001$). Other control measures (use of repellents, mosquito coils, vaporizing mats, closing of windows and doors in the evening, vapours of aromatic plants, and burning rubbish outside houses) accounted for 8.9% (17 / 200). Detailed descriptions will be presented only for the four main measures.

a) Smoke

Smoke was produced from a small fire, made from different materials (Table 5.5), placed in the bedrooms for approximately five minutes (Figure 5.7). This method of control repels sandflies for approximately two hours according to the information given by some of the householders. This measure has the longest history of use; the median number of years of use of smoke was 18.2 years ($q_{25} = 7.2$ years, $q_{75} = 31.2$ years). Several materials were used as fuel for smoke (Table 5.5) and commonly mixes of materials. The most common material was parts of aromatic plants, 51.4% (54 / 105),

Table 5.4 Control measures against sandflies practised by the householders in the three sampled villages. Original question: "Which measures to avoid sandfly biting have been practised by your family in this house?"

Type of control	Village							
	La Troja (n= 63)		Brasilia (n= 78)		El Cedral (n=59)		Total (n= 200)	
	No.	%	No.	%	No.	%	No.	%
Smoke	30	47.6	55	70.5	39	66.1	124	62.0
Bednets	11	17.5	30	38.5	32	54.2	73	36.5
House spraying with insecticides	32	50.8	18	23.1	16	27.1	66	33.0
House spraying with non-insecticidal substances	24	38.1	14	18.0	8	13.6	46	23.0
Others	4	6.4	10	12.8	3	5.1	17	8.9

(n): Refers to the number of householders who undertook some control measure.

Table 5.5 Selection of fuels used to make smoke for sandfly control in the three sampled villages.

Fuel type	Village							
	La Troja (n= 29 ^a)		Brasilia (n= 43 ^b)		El Cedral (n= 33 ^c)		Total (n= 105)	
	No.	%	No.	%	No.	%	No.	%
Aromatic plants (<i>Citrus spp.</i> , <i>Pinus spp.</i> , <i>Eucalyptus spp.</i> , and medicinal herbs)	11	37.9	17	39.5	26	78.8	54	51.4
Any plant	8	27.6	17	39.5	9	27.3	34	32.4
Manure (from cow or horse)	17	58.6	11	25.6	2	6.1	30	28.6
Coffee (pods and ground)	2	6.9	13	30.2	5	15.2	20	19.1
Inflamable rubbish	1	3.5	8	18.6	2	6.1	11	10.5
Other (debris from other plant parts)	2	6.9	3	7.0	2	6.1	7	6.7

^a One missing data point was not included; ^b Twelve missing data points were not included; ^c Six missing data points were not included; (n): refers to the total number of householders who used smoke as control measure for sandflies.



Figure 5.7 Smoke, made of *Citrus sp.* leaves, inside a bedroom used to repel sandflies (El Cedral village, Neiva municipality). Photo by Raul Pardo.

followed by any kind of plant, 32.4% (34/105), manure (from cow or horse), 28.6% (30 / 105), and coffee (pods or ground), 19.1% (20 / 105).

The use of materials, in addition to aromatic plants, differed by village according to the availability of resources (Table 5.5). In La Troja, the use of manure was significantly higher, 58.6% (17 / 29), than the use of any plant, 27.6% (8 / 29) ($X^2_{(1)} = 4.50$, $p = 0.034$), inflammable rubbish, 3.5% (1 / 29), ($X^2_{(1)} = 18.13$, $p < 0.001$) or coffee (pods or ground), 6.9% (2 / 29) ($X^2_{(1)} = 15.34$, $p < 0.001$). No significant difference was found between the use of manure, 58.6%, and aromatic plants, 37.9% (11 / 29) ($X^2_{(1)} = 1.73$, $p = 0.188$). In Brasilia, the use of parts of aromatic plants, and any plant, was the most common, both 39.5% (17 / 43), but there was no significant difference in the use of these materials ($X^2_{(4)} = 6.65$, $p = 0.156$). Finally, in El Cedral, parts of aromatic plants were the most common material used as fuel, 78.8% (26 / 33),

significantly more than any plant, 27.3% (9 / 33) ($X^2_{(1)} = 15.57, p < 0.001$), coffee (pods or ground), 15.5% (5 / 33) ($X^2_{(1)} = 18.4, p < 0.001$), and either manure or inflammable rubbish, both 6.1% (2 / 33) ($X^2_{(1)} = 26.7, p < 0.001$).

b) Bednets

Thirty seven percent (73 / 200) of householders who practised any control reported the use of bednets. The median time of use was 3.7 years ($q_{25} = 2.7$ years, $q_{75} = 6.2$ years). This shows that the acceptance of bednets by the inhabitants was high. In some cases, it was observed that householders made their own bednets with fragments of sheets and other fabrics, in spite of the possible discomfort caused by the reduction in flow of air inside the bednet (Figure 5.8). A total of 129 bednets were recorded in the three study villages; 16 bednets in La Troja, 56 bednets in Brasilia, and 57 bednets in El Cedral. Only 57% (74 / 129) of these bednets had a narrow mesh size (< 1 mm) appropriate as a physical barrier for sandflies. None of the bednets, with the exception of some nets treated once in 1999 in El Cedral (See Chapter 3, section 3.4), were impregnated with insecticide. Bednets did not cover all members of the family. There was a mean number of 0.41 (95% C.I.: 0.33 - 0.50) bednets / person / house.

c) House spraying with insecticides

House spraying had been used for a median time of 6.2 years ($q_{25} = 2.7$ years, $q_{75} = 11.2$ years). The most common insecticides were domestic insecticides, which were used by 94% of householders (62 / 66) and comprised a variety of brands. Eleven percent (7 / 66) of householders reported the use of agricultural insecticides (e.g. malathion, fenitrothion). No spraying campaign was reported to have been carried out for the last two years, prior to the present study, by the Health Services of the three municipalities which are responsible for health in the study villages.

d) House spraying with non-insecticidal substances

House spraying with non-insecticidal substances has been used for a median time of 7.2 years ($q_{25} = 2.2$ years, $q_{75} = 15.2$ years), similar to the time reported for spraying with



Figure 5.8 Home made bednet assembled out of fragments of different fabrics (Brasilia village, Tello municipality). Photo by Raul Pardo.

insecticides. Almost all the householders, who sprayed with non-insecticides, used petroleum derivatives (e.g. gasoline, kerosene, and other fuels), 95% (42 / 46); and a few, 13.0% (6 / 46), used creolin. It was reported that these substances are used as repellents for sandflies because of their strong smell.

5.3.2.2 Comparison of control measures by village

The frequency with which each control measure was practiced varied between villages (Table 5.4): smoke ($X^2_{(2)} = 8.35$, $p = 0.015$), bednets ($X^2_{(2)} = 17.99$, $p < 0.001$), house spraying ($X^2_{(2)} = 13.42$, $p < 0.001$), and house spraying with non-insecticidal substances ($X^2_{(2)} = 12.20$, $p = 0.002$). Smoke and bednets were more used in El Cedral and Brasilia; while smoke and both types of sprays (with insecticide and with non-insecticidal substances) were more common in La Troja.

5.3.2.3 Frequency of use of the control measures

Table 5.6 shows the frequency of use of the different control measures. An overwhelming majority of the householders said they used the control measures only during the "season of sandfly abundance": smoke, 91.1% (113 / 124); house spraying with insecticide, 86.2% (56 / 65), house spraying with non-insecticidal substances, 90.7% (39 / 43), and bednets, 76.4% (55 / 72). It was notable that 22.2% (16 / 72) of householders reported that they use bednets all year round.

The majority of householders, 56.4% (97 / 172), reported that the "season of sandfly abundance" was during both the two dry seasons, and a further 27.9% (48/172) reported that the season was either the first or second dry season. Neither percentage varied significantly with village, gender or age ($X^2_{(2)} = 2.65$, $p = 0.266$, and $X^2_{(2)} = 0.25$, $p = 0.882$, respectively) (Figure 5.9).

5.3.3 Economic status and the practice of the control measures

5.3.3.1 Relationship between house features of economic status and control measures

Information gathered from different sources indicates that bednets and house spraying with insecticides were measures of high cost, whereas house spraying with non-insecticidal substances and smoke were measures of low cost. According to information from the main market in Neiva city, where many people from the study area shop, the cost (in US dollars) of a narrow mesh size bednet is US\$ 4.30. The cost of house spraying with domestic insecticides (according to information collected by a health worker from Tello municipality) ranges from \$1.00 to \$1.30 (bottle of 230 cm³), and householders who use insecticides could spend up to \$18 / year specifically for sandfly control. The cost of non-insecticidal substances was considered as minimal because these substances (e.g. creolin, kerosene, gasoline) are cheaper than insecticides and they are bought mainly for other uses (cleaning, fuels for cooking and engines) rather than sandfly control. Smoke was considered also as a measure of minimal cost because the fuels (e.g. parts of different plants and manure) are obtained for free around the houses.

Table 5.6 Frequency of use of control measures for sandflies practised by the householders in the three villages sampled. Original question: "How often do you use the control measure?"

		Frequency of use													
Village	Type of control	All time		Season of high abundance of sandflies		Rainy season		Dry season		Once or twice a week		Occasionally		Other	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
La Troja	Smoke (n= 30)	1	3.3	26	86.7	0		1	3.3	2	6.7	0		0	
	House spraying with insecticides (n= 32)	1	3.1	28	87.5	0		0		1	3.1	1	3.1	1	3.1
	House spraying with non-insecticides (n= 22 ^b)	1	4.5	20	90.9	0		0		0		0		1	4.5
	Bednets (n= 10 ^a)	3	30.0	7	70.0	0		0		0		0		0	
	Others (n= 3 ^a)	1	33.3	2	66.8	0		0		0		0		0	
Brasilia	Smoke (n=55)	1	1.8	51	92.7	0		0		0		2	3.6	1	1.8
	House spraying with insecticides (n= 18)	0		15	83.3	1	5.6	0		0		2	11.1	0	
	House spraying with non-insecticides (n= 13 ^b)	0		12	92.3	0		0		0		1	7.7	0	
	Bednets (n=30)	7	23.3	23	76.7	0		0		0		0		0	
	Others (n= 10)	2	20.0	7	70.0	1	10.0	0		0		0		0	
El Cedral	Smoke (n= 39)	0		36	92.3	0		0		0		2	5.1	1	2.6
	House spraying with insecticides (n= 15 ^a)	1	6.7	13	86.7	0		0		0		1	6.7	0	
	House spraying with non-insecticides (n= 8)	1	12.5	7	87.5	0		0		0		0		0	
	Bednets (n= 32)	6	18.8	25	78.1	0		0		0		0		1	3.1
	Others (n= 3)	1	33.3	1	33.3	0		0		0		0		1	33.3
Total	Smoke (n= 124)	2	1.6	113	91.1	0		1	0.8	2	1.6	4	3.2	2	1.6
	House spraying with insecticides (n=65)	2	3.1	56	86.2	1	1.5	0		1	1.5	4	6.2	1	1.5
	House spraying with non-insecticides (n=43)	2	4.7	39	90.7	0		0		0		1	2.3	1	2.3
	Bednets (n=72)	16	22.2	55	76.4	0		0		0		0		1	1.4
	Others (n=16)	4	25.0	10	62.5	1	6.3	0		0		0		1	6.3

^a One missing data was not included, ^b two missing data were not included.

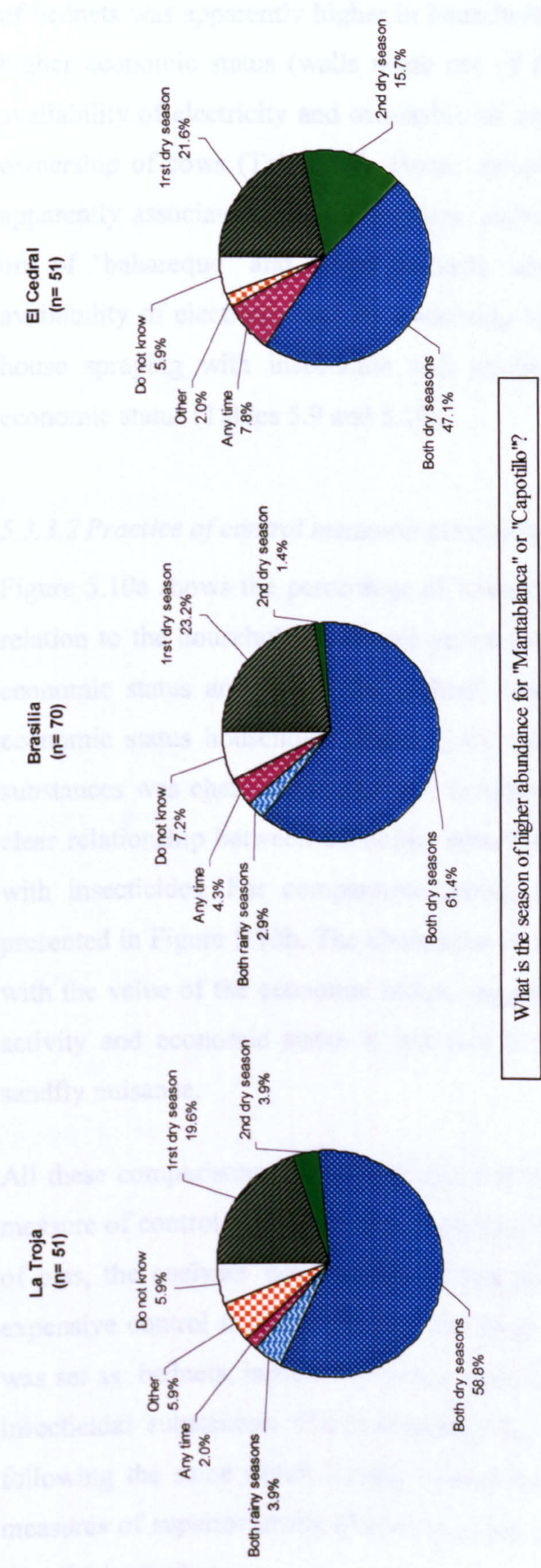


Figure 5.9 Season of the highest abundance of sandfly by village according to the householder who answered "season of sandfly abundance" to the question on frequency of use of the measures of control for sandflies (n = 172).

Tables 5.7 to 5.10 show the relationship between the house features selected to reflect economic status and the frequency of practising each of the control measures. The use of bednets was apparently higher in households with features considered as reflecting a higher economic status (walls made out of brick, presence of ceiling, few openings, availability of electricity and ownership of pigs and equines) with the exception of the ownership of cows (Table 5.7). House spraying with non-insecticidal substances was apparently associated with the features reflecting lower economic status (walls made out of "bahareque" and others material, absence of a ceiling, many openings, no availability of electricity and no ownership of pigs and cows) (Table 5.8). The use of house spraying with insecticide and smoke were, apparently, not associated with economic status (Tables 5.9 and 5.10).

5.3.3.2 Practice of control measures according to the Index of economic status

Figure 5.10a shows the percentage of householders practising each control measure in relation to the household economic status index (where "0" corresponds to the lowest economic status and "8" to the highest). Bednets were most frequent amongst high economic status households (index ≥ 4); while house spraying with non-insecticidal substances was characteristic of low economic status households (≤ 2). There was no clear relationship between economic status index and either smoke or house spraying with insecticides. For comparative purposes, the indoor abundance of sandflies is presented in Figure 5.10b. The abundance of *L. longiflocosa* females was not associated with the value of the economic index, suggesting that any association between control activity and economic status is not due to any confounding impact of the level of sandfly nuisance.

All these comparisons ignored the fact that many households practised more than one measure of control (as was shown in section 5.3.2.1). To deal with this potential source of bias, the analyses were repeated categorising householders according to the most expensive control measure (if any) that they used (Table 5.11). The order of expense was set as: bednets, insecticidal house spraying, smoke, and finally spraying with non-insecticidal substances. The socioeconomic index for each measure was computed, following the same order, having excluded the data points of the householders with measures of superior levels of cost (e.g. the index for house spraying with insecticides was obtained after

Table 5.7 The relationship between the use of bednets and some house features reflecting household economic status (the first category listed in each variable reflects lower economic status).

Variable	n	Bednets				$X_{(1)}^2$	p
		Yes	%	No	%		
Wall type							
"Bahareque" or others	159	51	32.1	108	67.9		
Brick	38	21	55.3	17	44.7	6.15	0.013
Wall cracks							
Many (>30%)	34	12	35.3	22	64.7		
None or few (0 - 30%)	161	60	37.3	101	62.7	<0.01	0.983
Presence of ceiling							
No	67	19	28.4	48	71.6		
Yes	131	53	40.5	78	59.5	2.31	0.129
Total openings							
Many (>5.8 m ²)	58	17	29.3	41	70.7		
None or few (0 - 5.8 m ²)	125	48	38.4	77	61.6	1.06	0.303
Presence of electricity							
No	16	4	25.0	12	75.0		
Yes	176	68	38.6	108	61.4	0.65	0.418
Presence of pigs							
No	143	50	35.0	93	65.0		
Yes	49	20	40.8	29	59.2	0.32	0.574
Presence of cows							
No	172	63	36.6	109	63.4		
Yes	21	6	28.6	15	71.4	0.24	0.627
Presence of equines							
No	153	52	34.0	101	66.0		
Yes	43	19	44.2	24	55.8	1.1	0.294

Table 5.8 The relationship between the use of house spraying with non-insecticidal substances and some house features reflecting household economic status (the first category listed in each variable reflects lower economic status).

Variable	n	House spraying with non-insecticidal substances				$X_{(1)}^2$	p
		Yes	%	No	%		
Wall type							
"Bahareque" or others	159	44	27.7	115	72.3		
Brick	38	2	5.3	36	94.7	7.40	0.006
Wall cracks							
Many (>30%)	34	4	11.8	30	88.2		
None or few (0 - 30%)	161	40	24.8	121	75.2	2.05	0.152
Presence of ceiling							
No	67	19	28.4	48	71.6		
Yes	131	27	20.6	104	79.4	1.09	0.297
Total openings							
Many (>5.8 m ²)	58	19	32.8	39	67.2		
None or few (0 - 5.8 m ²)	125	24	19.2	101	80.8	3.33	0.068
Presence of electricity							
No	16	6	37.5	10	62.5		
Yes	176	39	22.2	137	77.8		0.214
Presence of pigs							
No	143	37	25.9	106	74.1	1.36	0.244
Yes	49	8	16.3	41	83.7		
Presence of cows							
No	172	41	23.8	131	76.2		
Yes	21	4	19.0	17	81		0.788
Presence of equines							
No	153	34	22.2	119	77.8		
Yes	43	11	25.6	32	74.4	0.07	0.797

Table 5.9 The relationship between house spraying with insecticides and some house features reflecting household economic status (the first category listed in each variable reflects lower economic status).

Variable	n	House spraying with insecticides				$X_{(1)}^2$	p
		Yes	%	No	%		
Wall type							
"Bahareque" or others	159	52	32.7	107	67.3		
Brick	38	14	36.8	24	63.2	0.09	0.769
Wall cracks							
Many (>30%)	34	13	38.2	21	61.8		
None or few (0 - 30%)	161	51	31.7	110	68.3	0.29	0.590
Presence of ceiling							
No	67	19	28.4	48	71.6		
Yes	131	47	35.9	84	64.1	0.81	0.367
Total openings							
Many (>5.8 m ²)	58	22	37.9	36	62.1		
None or few (0 - 5.8 m ²)	125	34	27.2	91	72.8	1.67	0.196
Presence of electricity							
No	16	7	43.8	9	56.3		
Yes	176	55	31.3	121	68.8	0.55	0.457
Presence of pigs							
No	143	38	26.6	105	73.4		
Yes	49	23	46.9	26	53.1	6.07	0.014
Presence of cows							
No	172	53	30.8	119	69.2		
Yes	21	10	47.6	11	52.4	1.70	0.192
Presence of equines							
No	153	51	33.3	102	66.7		
Yes	43	14	32.6	29	67.4	0.01	0.930

Table 5.10 The relationship between the use of smoke and some house features reflecting household economic status (the first category listed in each variable reflects lower economic status).

Variable	n	Smoke				$X_{(1)}^2$	p
		Yes	%	No	%		
Wall type							
"Bahareque" or others	159	96	60.4	63	39.6	0.19	0.667
Brick	38	25	65.8	13	34.2		
Wall cracks							
Many (>30%)	34	26	76.5	8	23.5	3.15	0.076
None or few (0 - 30%)	161	94	58.4	67	41.6		
Presence of ceiling							
No	67	44	65.7	23	34.3	0.47	0.497
Yes	131	78	59.5	53	40.5		
Total openings							
Many (>5.8 m ²)	58	34	58.6	24	41.4	0.29	0.593
None or few (0 - 5.8 m ²)	125	80	64.0	45	36.0		
Presence of electricity							
No	16	9	56.3	7	43.8	0.07	0.787
Yes	176	111	63.1	65	36.9		
Presence of pigs							
No	143	91	63.6	52	36.4	0.41	0.523
Yes	49	28	57.1	21	42.9		
Presence of cows							
No	172	109	63.4	63	36.6	0.55	0.458
Yes	21	11	52.4	10	47.6		
Presence of equines							
No	153	93	60.8	60	39.2	<0.01	0.951
Yes	43	27	62.8	16	37.2		

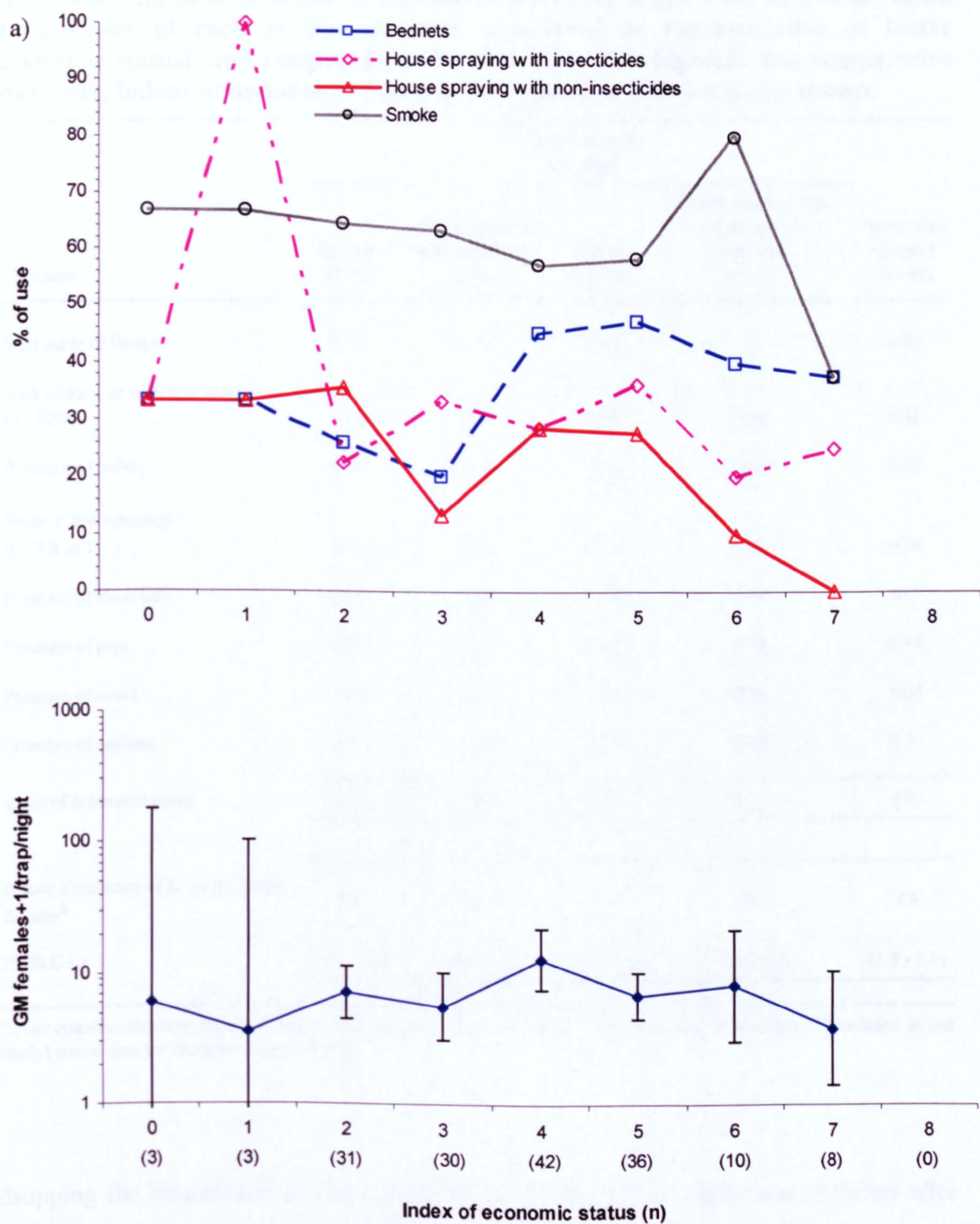


Figure 5.10 Relationship between the value of the economic status index (0 = lowest, 8 = highest) and (a) percentage of use of control measures against sandflies, (b) indoor sandfly abundance (error bars are 95% C.I.).

Table 5.11 Index of economic status for households practising each of the control measures (and none of the more expensive measures). Index = the sum of the mean proportions of each of the variables considered as representative of better economic status), and ranging from "0" lowest, to "8" highest). For comparative purposes, indoor abundance of *Lutzomyia longiflocosa* females is also shown.

Variable	Control measure (n= 196) ^a				
	Bednets (n= 73)	House spraying with insecticides (n= 46)	Smoke (n= 64)	House spraying with non-insecticidal substances (n= 13)	No control measure (n= 44)
Wall made of Bricks	0.29	0.09	0.17	0	0.18
Wall without or with few cracks (0 - 30%)	0.83	0.77	0.78	1.00	0.80
Presence of ceiling	0.74	0.70	0.59	0.46	0.70
None or few openings (0 - 5.8 m ²)	0.74	0.60	0.73	0.42	0.68
Presence of electricity	0.94	0.88	0.90	1.00	0.89
Presence of pigs	0.29	0.37	0.15	0.08	0.19
Presence of cows	0.09	0.18	0.08	0.08	0.28
Presence of equines	0.27	0.22	0.19	0.08	0.34
Index of economic status	4.18	3.81	3.59	3.11	4.06
Indoor abundance of <i>L. longiflocosa</i> females ^b	5.5	4.6	8.2	20	2.4
(95% C.I.)	(3.7 - 7.9)	(2.5 - 8.0)	(5.4 - 12)	(7.6 - 49)	(1.3 - 3.9)

^a Four householders who reported other control measures were excluded; ^b Based on 232 houses (from 237 included in this study) where data for sandflies were available.

dropping the householders who used bednets; the index for smoke was obtained after dropping the householders who used bednets and house spraying with insecticides, and so forth). The highest indices of economic status were obtained for the use of bednets (4.2) and house spraying with insecticides (3.8) and the lowest for smoke (3.6) and house spraying with non-insecticidal substances (3.1). These results are consistent with the hypothesis that bednets and house spraying are control measures practised by relatively high economic status households; while smoke and spraying with

non-insecticidal substances are characteristic of lower economic status households. Hence, the findings indicate that household economic status affects the choice of control measure practised. However, householders who did not practise any control measure had a relatively high economic index (4.1), suggesting that economic status was not the limiting factor; instead it would appear that these households did not practise control as their houses had relatively low sandfly abundance, GM = 2.4 (1.3 - 3.9) s/LT/n, and hence low nuisance level. Analysis of the abundance of *L. longiflocosa* females indoors supports this hypothesis as the female abundance in the group which did not apply any control measure was the lowest, (Table 5.11). It was also notable that the highest abundance of *L. longiflocosa* occurred in houses with apparently the lowest economic status, GM = 20 (7.6 - 49) s/LT/n.

5.4 DISCUSSION

Although previous studies of KAP on leishmaniasis have included entomological aspects, I am unaware of any prior study that also included measures of sandfly abundance. The inclusion of questions on the control of the sandfly vector, as well as of the disease, allowed a more complete analysis of the general understanding of the disease and its control by the community. However, the present study may have some limitations: representativeness of the interviewees, response and recall bias. It was considered that the interviewees were representative of the KAP of all the study villages in spite of some detected differences, specifically gender. Most of the interviewees were women, 58% (probably because the interviews were conducted during the day, when most of men were involved in outdoor activities) while the study population was biased to men (56%). However, as no effect by gender was found in any of the questionnaire comparisons, this bias may not be important. The study was also restricted to adults (> 18 years old), excluding younger age groups which could have provided a complete picture of the KAP of the households. As well as the practical advantages of interviewing adults, the rationale was that it is the adult population who are responsible for the health of their families, including the practices of control. Finally, this study ignored other important aspects of the KAP of the study population such as a description of clinical features of the disease, treatment seeking, and treatment adherence. It was considered that these aspects were covered in sufficient detail by a previous study carried out within the study area (Nicholls *et al.*, 1998).

5.4.1 Knowledge of CL, sandflies and control

Taking into account the knowledge of disease, sandflies, the role of sandflies as vectors, and the practice of control measures against CL, it could be said that the "integral understanding" of CL is low in the study area. This was evident in the low knowledge of the role of sandflies in the transmission, 29% of the total householders, and in the low use of measures to control CL, which was reported by only 30% of total householders.

In spite of the low "integral understanding" of CL, the householders had a good knowledge of some of the different components of the disease and its control. It was remarkable that 86% of householders recognized CL, and everyone, with the exception of five people, knew sandflies. These results are in concordance with the findings of the previous study on KAP within the study area, where 83% of interviewees could describe the clinical features of the disease, and more than 80% recognized sandflies (Nicholls *et al.*, 1998). That study also found that 87% of householders said CL is a serious disease, 99% said that when the disease appears the patient must go to the physician, and most considered glucantime as the appropriate treatment, although some of them reported the use of traditional treatments. This relatively high knowledge of the disease, including the findings in the present study, could be explained in part by the experience of the recent epidemic (1993 - 1996) and some educational campaigns which were carried out by the Huila Health Services (SSDH) at that time. The high degree of knowledge of sandflies could also be due to the high human-sandfly contact indoors in the study area. Evidence of this comes from the results of the Risk Factor study (Chapter 3) where a high indoor activity of *L. longiflocosa* was detected: sandflies were collected in 86% of all houses, including those in the present study. In areas where the human-sandfly contact is low, the percentage of inhabitants who recognize sandflies is usually lower. For example, in the coffee plantations in Minas Gerais, Brazil where sandfly abundance was apparently low, only 23% of interviewees knew sandflies (Alexander *et al.*, 2002).

In relation to the knowledge of the role of sandfly in the transmission of CL, the results in this study was lower, 32%, than in the previous study by Nicholls *et al.* (> 70%). This discrepancy could be explained by differences between the questionnaires used in the two studies. In the previous study, the question "Which of these insects could transmit CL?" (when a sandfly and a bed bug were shown to the interviewees) could be misleading, as the interviewee was forced to give an answer based on a supposition.

Even if he or she did not know the answer, the person could choose the sandfly as they are more familiar with this insect (it seems that bugs are much less abundant than sandflies in the study area). In the present study, a more direct and open question was used: "Which disease is caused by "Capotillo" or Mantablanca?". It is unlikely that householders could be misled by this question, by confusing sandflies with other related insects because sandflies are well known for their biting habits and their close contact with humans inside houses.

The apparently low practice of control for CL in this study, 35%, was similar to that found in the previous study (Nicholls *et al.*, 1998), where only 48% of householders said they knew any type of control for CL, but is higher than that in two other areas in the Sub-Andean region studied by Nicholls *et al.* (villages of the departments of Norte de Santander and Cundinamarca). Knowledge of control measures was reported by 18% of interviewees in Norte de Santander and 19% in Cundinamarca.

A remarkable 82% of households in this study reported sandfly control measures, much more than the 43% reported in the Brazilian study of a CL endemic population (Santos *et al.*, 2000). Again, this is consistent with the perception of significant indoor sandfly nuisance, due to high indoor sandfly abundance, in Huila. Indeed more than twice as many households practised control measures to deal with sandfly nuisance (82%) than to deal with CL (35%). However, there was evidence that CL control measures were significantly more likely to be practised by those with a knowledge of the role of sandflies in transmission (51%). Hence, it is possible that in those persons who know the role, the sandfly is perceived as a real danger to the health of the family. It is also possible that the person who knows the role also has more access to education about the disease, causing them to practise control measures against CL. Further evidence for an association between knowledge of the role of sandflies and CL control activities was reported in the comparative study of Nicholls. In Huila, where > 70% interviewees claimed to know the role of sandflies (but see above), 48% of householders practised control for CL. In contrast, < 20% of interviewees in two other departments knew the sandfly role, and a similarly low percentage of householders practised CL control.

5.4.2 The practice of control measures

A relatively small number of control measures for sandflies were practised in the study area: a) smoke, b) bednets, c) house spraying with insecticides, and d) house spraying with non-insecticidal substances. These were the same as used for control of CL. As the most widely used practice was smoke (practiced by 62% of householders), the following discussion will deal with this in some detail and the other practices will be treated briefly.

The use of smoke is probably the first mosquito repellent used by humans. Fresh or dry leaves, bark, flowers and fruits of different plants and others materials have been used to produce smoke to repel mosquitoes in China and Japan (Laird and Miles 1983). In Gambia, wood and resin of aromatic plants are sold to be burned as mosquito repellents (Curtis *et al.*, 1990). In India smoke from leaves of “nochi” (*Vitex negundo*) has also been reported to repel mosquitoes from houses (Rozendaal 1997). Plants used to make smoke could also be used in other traditional ways such as rubbed on the skin or hung in the house. These plants could contain repellent or insecticidal compounds. Around 1200 plant species has been listed in the literature as of potential insecticidal value (Roark 1947). Smoke probably works in several ways (Moore and Lenglet 2003): a) the smoke may disguise human kairomones and disrupt convention currents essential in mosquito host location; and b) burning may release repellent, irritant or insecticidal molecules. Examples of plants which contain repellents (names within brackets) are: *Eucaliptus maculata citronella* (p-menthane-3, 8 diol), *Cymbopogon martini martini* (geraniol), and *C. nardus* (citronella). Plants which contain insecticidal compounds are: *Crysantemum spp.* (pyrethrins), *Derris eliptica* (rotenone), and *Nicotiana spp.* (nicotine) (Curtis *et al.*, 1990). Amongst the most important components obtained from plants are the pyrethrins which were the base for the synthetic pyrethroids.

In spite of the wide use of repellent and insecticide components extracted (or synthesized) from many plants for commercial purposes, there is little literature concerning the effectiveness of traditional preparations such as smoke, in particular on sandflies. For old world species of sandflies, in China it was reported that indoor smoking with tobacco, pyrethrum or artemisia kept sandflies away for 1 – 2 days (Lu *et al.*, 1955). In Ethiopia, the absence of *Phlebotomus longipes* in some buildings was attributed, in part, to the smoke indoors (mainly from cooking fires), and their

abundance in bedrooms to the absence of smoke (Foster 1972). For new world species, Herrer (1956) pointed out that smoke in houses is an irritant for sandflies; and Llanos-Cuentas (1994) indicated that in some endemic areas for leishmaniasis the households produce smoke indoors during the periods of high density of sandflies as a measure of protection.

In Huila, this traditional measure of control, in addition to being the most common, was the measure with the longest history of use by the householders (used for an average of 18 years approximately, and known for more than 30 years). Its popularity could be due mainly to its low cost, as the necessary materials to make the smoke are obtained practically free of charge and near the house. Use of smoke as the main control measure by householders against sandfly vectors of CL has also been reported in Brazil (Santos *et al.*, 2000), where 88% of those who used any form of control against biting arthropods (among them sandflies) reported the use of smoke. In Huila, there was no strong preference for any specific type of fuel with a better repellent effect. However, aromatic plants (e.g. *Citrus spp.*, *Pinus spp.*, and *Eucalyptus spp.*) were the most common fuel, used by 51% of householders. A previous study in Guinea Bissau, West Africa, has shown that outdoor smoke made from leaves of *Eucalyptus sp.* had a repellency of 72% against mosquitoes (Palsson and Jaenson 1999). Smoke of other plants used indoors has also shown a repellent effect. Nicholson and Lines, cited by Curtis *et al.* (1991), showed that indoor biting by *Culex quinquefasciatus* was reduced up to ten times when *Hyptis suaveolens* was smouldered on a charcoal fire; they also showed that the smoke of this plant was more effective than that of grass, coconut husks, or neem.

Short-term effect measures, such as smoke, could have some impact on CL if it is practised continuously during the time of highest risk of transmission. Previous studies have shown that smoke could have a protective effect. In a case-control study carried out in Santiago del Estero, Argentina, the use of smoke reduced the risk of CL (OR = 0.3, $p = 0.033$) when factors for indoor transmission were considered, but it was not significant when other factors of peridomicile and human behaviour were taken into account (Yadon *et al.*, 2003). In another case-control study, carried out in two regions of Peru, smoke in houses was identified as a protective factor (Llanos-Cuentas 1994).

Whilst smoke may be effective as a short-term control measure against sandflies, it is also clear that smoke also has some negative impact: not just unpleasant odours, but also a cause of ill health. Indoor air pollution (IAP), as a result of the combustion of fuels used in cooking fires, especially inside houses, has been implicated as a risk factor for several diseases, related mainly with the respiratory system such as acute respiratory infections, chronic obstructive pulmonary disease and asthma among others (Ezzati and Kammen 2002). Although, exposure time to smoke to control sandflies is supposed to be shorter than the exposure to a cooking fire, it is probable that the concentration of particulate matter in the smoke could be much higher than the concentration produced by a cooking fire, particularly immediately after the activity is carried out. Taking into account the potential effectiveness of some plants as repellents and the extended use of smoke by the householders within the study area, further research is needed to test the potential repellent effect of different local plants as fuels (or in another ways of application such as rubbed on the skin). Smoke has the advantages that it is easily available, inexpensive and already used by families as a control measure. These features could help to guarantee the sustainability of a control program. Finally, it is crucial that future studies evaluate the possible negative effects that smoke could have on human health.

The relative costs and benefits of using smoke to protect against sandflies have yet to be explored, but there is evidence that untreated bednets can help protect against sandflies (Chapter 4) and leishmaniasis if they are used properly. The use of untreated bednets was identified as a protective factor against VL transmitted by *P. argentipes* in Bangladesh (Bern *et al.*, 2005) and Nepal (Bern *et al.*, 2000). Amongst householders in Huila who practised any form of control, the use of untreated bednets was relatively common (37%), indicating relatively high acceptance in the population in spite of their apparent recent adoption (median time of use, 3.7 years). Attempts to introduce this control measure were carried out recently by the Neiva Health Service, and bednets were delivered in some villages, including El Cedral. Nevertheless, it appears that only a very few were delivered within the study area (Chapter 3, section 3.4), so they would not have significantly effected the results of the study. Indeed, there was no significant difference in the use of bednets between El Cedral and Brasilia (54% and 39%, respectively; $\chi^2_{(1)} = 2.77, p = 0.096$).

Given the relatively low coverage, 0.41 bednets/person/house, and the presumed lower effectiveness of around half of the bednets, 43%, which had a wide mesh size (> 1 mm) that does not provide a complete physical barrier, it seems likely that the current net usage patterns are providing relatively little protection against CL. The choice of a wide mesh net by the Huila study population appears to be due to access, and not because of high nocturnal temperatures (which can make narrow mesh nets uncomfortable). Daily temperatures in the sub-Andean region of Colombia present large variations, 10°C or more, with a mean temperature of 20°C at 1500 m a.s.l. Indeed, some people reported that narrow mesh bednets were preferable, precisely because they kept them warm and protected them from the cold during the night (Chapter 4, section 4.5.2.4).

Bednets are a relatively novel intervention in this region, compared to house spraying. The median time of use for spraying with insecticide was 6.2 years, and for non-insecticidal substances 7.2 years. These dates coincide with the last epidemic of CL (1993 - 1996), where the main control, carried out by the departmental and municipal Health Services, was the indoor and outdoor spraying of all houses in the epidemic villages. This intervention could have reinforced the use of these measures by householders.

The great majority of householders used control measures for sandflies only during the seasons of high sandfly abundance, which they reported as the two dry seasons. This provides further evidence that control activities are stimulated by the presence of sandfly nuisance – i.e. during the sandfly season. Preliminary results of an ongoing study on seasonal abundance within the study area (A. Carvajal, personal communication) are consistent with the interviewees opinions that sandflies are most abundant in the dry season - at least for *L. longiflocosa*, the most abundant sandfly species. The potential magnitude of the indoor sandfly nuisance during the dry seasons is provided by the data on indoor sandfly numbers described in Chapter 4 (section 4.5.2.1). Amongst 16 houses (with relatively high sandfly abundance), the GM number of *L. longiflocosa* females was 43 (24 - 80) f/LT/n and 50 (23 - 111) f/LT/n during the first (sampling in January) and second (sampling in July) dry seasons, respectively.

It is possible that the control measures practised by householders had some effect (at least temporally) as people continue to implement them (particularly smoke and house spraying with non-insecticidal substances). Nevertheless, no statistically significant reduction in sandfly abundance or CL prevalence could be demonstrated when the four main measures of control were tested by multivariate analysis (Chapter 3, section 3.5.4). The possible effectiveness of the control measures for sandflies needs to be investigated more thoroughly as the present study evaluated only the possible cumulative (long term) effect of the control measures on the sandfly population and CL cases, and provisions were taken to avoid any application of control measures during the nights of the sandfly sampling.

5.4.3 Relationship between economic status and control

It appears that the economic status of the householders limits the practice of some control measures. Households who used bednets or house spraying with insecticides tended to have a relatively high economic status (4.2 and 3.8, respectively), compared to those households who only applied relatively cheap measures, i.e. smoke and house spraying with non-insecticidal substances (3.6 and 3.1). While all families seemed to be able to afford to use either smoke or non-insecticidal substances, access to nets and insecticides was apparently limited by affordability. Evidence of the same type of economic limitations on the use of measures of control was found in the study of Santos *et al.* (2000) in an area endemic for CL in Brazil. Their results suggested that families with lower incomes (less than three minimum Brazilian salaries) practised less control measures which demand a high expenditure, 2% (3 / 142), compared with households with higher incomes (more than three minimum salaries), 15%. Nevertheless, no statistically significant differences were found. What was clear in the cited study was that the families with the lowest incomes did not practise any measure of control which involved expenditure. Although the results of the present study are not definitive, there is enough evidence to indicate that economic limitations are a factor which should be considered in the implementation of any control programme in the study area.

5.4.4 Village differences

In spite of some similarities which are shared by the three study villages, such as a high knowledge of CL (> 79%), sandflies (> 97%) and practice of sandfly control (> 77%), there are also some clear differences between villages. These differences are mainly related with knowledge of the sandfly role in disease transmission and the practice of control measures of CL.

There is no obvious explanation why the knowledge of sandfly role was significantly lower (16%) in Brasilia compared with the La Troja (33%) and El Cedral (44%). The differences could occur because of a different level of education of the householders. Householders with low education could be reluctant to believe the information given by the Health Services about CL transmission. Another possibility is that the educational campaigns given during the epidemic time by the SSDH with the support of the municipal Health Services of Baraya (for La Troja), Tello (for Brasilia) and Neiva (for El Cedral) varied in intensity or coverage between villages. Village differences in the practice of control measures for CL, which was significantly lower in Brasilia (25%) compared with the other two municipalities (39% and 46%, for La Troja and El Cedral, respectively) could be the direct result of the different knowledge of the sandfly role in transmission between villages, which is apparently positively correlated with the practice of CL control (section 5.4.1.3). These geographic differences in the “integral understanding” of CL should be considered for a differential management in future control programmes within the study area.

Village differences in the relative use of alternative control measures against sandflies could be linked to differences in economical development, as it was shown that economic status could limit the practice of control (section 5.4.3). La Troja is the poorest village (Chapter 3, section 3.5.4.2), with a relatively low economic status (at least compared to El Cedral); and La Troja households were the most likely to practise house spraying with non-insecticides (38%, as compared with 18% in Brasilia and 14% in El Cedral), and least likely to use bednets (18% compared to 39% in Brasilia and 54% in El Cedral). However, the poor economic status of La Troja would not explain why it has the highest use of insecticide spraying, and the lowest use of smoke. Hence, the geographic patterns cannot be easily explained.

5.4.5 Conclusions

In conclusion, despite the relatively low "integral understanding" of CL and its transmission amongst the surveyed population, householders had a good knowledge of some of the different components of the disease and its control. The significant association between the knowledge of sandflies' role in transmission and the practice of CL control provides evidence that there is scope for impacting control activities by health educational campaigns. However, the remarkably high level of sandfly control as compared to CL control practised by the community shows how vector control promotion programmes need to account for community attitudes to both sandfly nuisance as well as the diseases they transmit. Finally, it appears that economic status limits the choice of control measure used. Hence, to reduce inequities in health status amongst CL endemic communities, it may be worth considering bednet subsidies for the lowest economic status households within the context of potential social marketing campaigns to widen bednet usage.

6 CONCLUSIONS AND FUTURE STUDIES

This thesis has provided overwhelming evidence (behavioural, epidemiological and distributional) indicating that *L. longiflocosa* is the principal vector of CL in Huila: (1) the significant positive association between indoor abundance of *L. longiflocosa* and household cumulative prevalence of CL (Chapter 3); (2) anthropophagy (Chapter 2); (3) high abundance and dominance by all collection methods (89% in Chapter 2); (4) high endophagy (86% of positive houses in Chapter 3); (5) the similarity between the altitudinal patterns of *L. longiflocosa* abundance and CL cumulative prevalence indoors (Chapter 3); (6) the spatial overlap of *L. longiflocosa* abundance with the area of highest CL incidence at municipality level and within villages (Chapters 2 and 3); and (7) the occurrence of an outbreak of CL in Algeciras municipality soon after the detection of a high abundance of *L. longiflocosa* in this municipality during the study in Chapter 2. *L. nuneztovari* seems to play, at most, a secondary vectorial role because: (1) the negative association between indoor abundance of *L. nuneztovari* and household cumulative prevalence of CL; (2) low abundance and dominance (4.6%); (3) relatively low endophagy (27% of positive houses); (4) the different altitudinal patterns of *L. nuneztovari* abundance and CL cumulative prevalence indoors; and (5) lack of association of *L. nuneztovari* abundance with the spatial distribution of CL at municipality level and within villages.

CL foci in Huila are limited to the north east municipalities on the Cordillera Oriental. Similar to other Andean regions, sandfly species (particularly *L. longiflocosa*) tend to be restricted to specific environments defined by their altitudinal ranges, and CL risk is therefore closely associated with altitude. The altitude of highest risk for CL according to the distribution of *L. longiflocosa* and CL cases reports is between 1500 – 1700 m a.s.l. . Very low or no risk is expected below 900 m a.s.l. . Also, as in other Andean regions, a major proportion of CL transmission in Huila department occurs indoors, although outdoors transmission also occurs. The main findings which support indoor transmission of CL are: (1) the positive association between indoor abundance of *L. longiflocosa* and household cumulative prevalence of CL, and (2) the endophagic

behaviour of this sandfly species - as indicated by LTC in Chapters 2, 3 and 4, and confirmed by (i) the evidence that indoor sandflies are a nuisance for householders, and (ii) the relatively high human landing rates recorded indoors (Chapter 4, section 4.3.1). Transmission away from houses is supported by the apparent increase in CL risk for adult males and by the high abundance of *L. longiflocosa* in forest and traditional coffee plantations. Hence, there is definitely a high intensity of human-sandfly contact within the study area both indoors and outdoors. Because the risk factor study focused on possible risk factors for indoor transmission, few data were collected on potential risk factors for outdoor transmission; the latter should be tested in the future (e.g. by a case-control study) to provide an unbiased comparison of the risks of exposure in different sites of CL transmission.

Although this study did not collect direct evidence on seasonality in disease transmission, the analysis of the seasonal distribution of cases during the last epidemic in Baraya municipality, and the report of seasonality in sandfly abundance by the community, both suggest that transmission rates are highest during the seasons of highest sandfly abundance (dry or low rain seasons). But this hypothesis needs further confirmation and studies on the parity status of the host-seeking sandfly populations.

L. longiflocosa is a dry season species of the sub-Andean region, the latter being characterised by a temperate climate, with a narrow ecological niche defined by clear limits of altitude and temperature, and associated with specific patterns of rainfall and soil. Within regions where these environmental conditions prevail, this sandfly species is most abundant in sites with a relatively high slope, protected from wind, and the presence of a well structured and complex forest (with several tree strata, high cover and high litter cover). Indoor abundance is additionally dependent on the number of people and dogs in the house, and by the density of houses and pasture around the house.

In contrast, *L. nuneztovari* is a generalist species with a less well defined preference for temperature, rainfall or soil conditions. Within its endemic region, abundance is highest in sites with relatively low slope, but is less influenced by protection from wind. Other risk factors include the presence of highly disturbed forest (few tree strata with low litter) located near human dwellings. Indoor abundance depends additionally on

variables related with house features (type of ceiling), its surrounding habitat (presence of banana plants and number of domestic animals) and household size.

Both *L. longiflocosa* and *L. nuneztovari* are forest species which seem to be “completely adapted” to traditional coffee plantations, presumably due to the presence of trees in this type of coffee plantations. *L. longiflocosa* and *L. nuneztovari* appear to have a “partial adaptation” to the two types of intensive coffee plantations, using these habitats apparently only for foraging activities. Adaptation to intensive coffee growing, particularly unshaded plantations, seems unlikely because the strong structural change caused by the loss of tree strata makes this habitat unsuitable for sandflies. Nevertheless, future adaptation of either *L. longiflocosa* or *L. nuneztovari* to intensive unshaded coffee growing areas cannot be discounted.

The occurrence of indoor transmission in the sub-Andean region of Huila department provides an opportunity for CL control by reducing the rate of sandfly bites inside houses. The choice of ITNs is proposed because: (1) the use of bednets (unlike residual insecticide house spraying) is not affected by the exophilic behaviour, which appears to characterise *L. longiflocosa* in this region (according to preliminary observations); (2) preliminary results (Chapter 4, section 4.2.1.1) suggest that peaks of high biting activity occur during the time (21:00 – 0:00 h) the inhabitants have retired to rest; (3) a relatively high percentage of households (37 %) already use (untreated) bednets; and (4) because householders reported additional benefits (protection from cold) which could increase acceptance.

The efficacy and effectiveness studies (Chapter 4) indicate that lambda-cyhalothrin (25 mg/m²) treated bednets may be useful for the control of *L. longiflocosa* in Huila department. The efficacy study showed that ITNs: (1) reduced the human biting rate by *L. longiflocosa* both inside and outside nets; (2) caused high immediate (because of knockdown effect) and 24 h mortality; and (3) had a protective effect on people located outside the bednet but in the same room. There was no convincing evidence for excito-repellency effects of the ITNs. Even untreated wide mesh nets seemed to provide significant protection, as compared to unprotected people outside nets. The effectiveness study showed that four months post-treatment for ITNs: (1) the residual effect of insecticide on nets was maintained; (2) outside-net landing rates were also still

significantly lower in rooms with nets compared with controls (rooms without nets); (3) light trap catches in rooms with people sleeping under nets contained less sandflies, a lower percentage of bloodfeds, smaller blood meals, and a lower HBI than in control houses. For reasons explained in Chapter 4, the observed reduction in sandfly numbers collected in rooms with ITNs (as measured either by light traps or by a combination of direct search and human catches) seems to reflect a true reduction in indoor transmission risk, as the LTC : HLC ratio outside nets was the same in both ITN and control houses. In contrast, the effect of house spraying on indoor sandfly biting rates was more confused. In sprayed houses, four months post-treatment: (1) the residual effect of insecticide on walls had significantly dropped, but still caused considerable mortality; (2) human landing rates were the same in sprayed and control houses; but unexpectedly (3) light trap catches in sprayed houses (as compared to control houses) also found less sandflies, a lower percentage of bloodfeds, smaller bloodmeals, and a lower HBI than in control houses. These latter results are best explained by the differential effectiveness of CDC light traps for catching blood-fed females in sprayed houses, and not by a real reduction caused by the insecticide (as the LTC : HLC ratio was much lower in sprayed houses than in control houses). The reduction in the insecticide residual effect after 4 months in the sprayed houses may have contributed to the lack of effectiveness of this treatment. Hence, at household level (not mass spraying, which was not evaluated in this study), the results indicate that house spraying is not effective for controlling *L. longiflocosa* – at least in Huila.

Based on their acceptability and their entomological effectiveness it is possible that the introduction of lambda-cyhalothrin treated bednets could be used to control CL within the study area and in other foci where *L. longiflocosa* is the primary vector. Amongst several factors to be considered in the implementation of ITNs, the results of this thesis highlight the following:

(1) Scale of the intervention, insecticide and variables to measure. Future interventions with ITNs could aim for high coverage of all the villages at risk of the epidemic area, where *L. longiflocosa* is abundant. This could provide a mass effect on vector abundance and survival rates (and hence on infection prevalence) in addition to the personal protection demonstrated in this thesis. Alternatively, a focal intervention could be more cost-effective, due to the observed highly aggregated distribution of

L. longiflocosa, demonstrating that people vary greatly in exposure and hence CL risk. This would suggest that ITNs should be targeting at those houses with the highest abundance of *L. longiflocosa*, in each village at risk. The risk factors for outdoors and indoors *L. longiflocosa* abundance detected in Chapters 2 and 3 could be used in the selection of households to target.

This study tested lambdacyhalothrin treated nets, but it may be more appropriate to implement long-lasting ITNs, with special treatment (added when manufactured or to already in use bednets) to ensure insecticide effectiveness persists over time and after many washes (Yates *et al.*, 2005; Graham *et al.*, 2005), and so eliminate the need for annual re-impregnation of nets (and hence improve sustainability). The study also identified how difficult it is to evaluate personal protection of bednets entomologically under natural usage conditions (i.e. true effectiveness). Future trials could exploit the recent findings that humans develop species-specific antibodies to sandfly saliva antigens, so that a reduction in sandfly exposure due to ITN implementation could be identified (in theory) by a reduction in antibody titres (Rohousova *et al.*, 2005; Louzir *et al.*, 2005). While the ultimate measure of effectiveness should be the impact on CL incidence, as disease rates are low and quite variable year-on-year, entomological indicators remain important, and evaluations could also record effects on infection rates (i.e. including subclinical infections).

(2) Education campaigns. The significant association detected between household knowledge of the role of sandflies in transmission and the practice of CL control indicates that health education campaigns could improve the acceptability and community involvement in control activities with ITNs. In these campaigns (akin to the ITN promotion programmes in Africa for controlling malaria) it is crucial to take into account the importance that the community gives to both sandfly nuisance as well as the diseases they transmit. The target population should be the young population because they comprise a large percentage (39% < 16 years old) of the whole population, they are the easiest to target in terms of better “disposition” to incorporate new knowledge, and the knowledge has more chance to persist in the long time. In addition, educational campaigns could be delivered by incorporating them into the schools’ curricula. Such measures should help to maintain a long-term community prevention programme.

(3) Effect of seasonality in control. If it is confirmed that transmission rates are especially high during or around the seasons of highest sandfly abundance, then the use of ITNs should be particularly encouraged during this period; i.e. health promotion resources should be focused when it is likely to have the greatest impact. Additionally, receptivity to control campaigns during this time is expected to be high because of the highest nuisance cause by sandflies.

(4) Subsidies to reduce inequities in health status. Given the indications that economic status limits the choice of control measure, it is necessary to consider bednets subsidies for the lowest economic status households to help widen bednet usage and reduce inequities in health status amongst CL endemic communities.

(5) Mesh size of the bednets. Finally, it should be pointed out that the use of a small mesh size (0.7 mm), which provided a significant physical barrier against sandfly entry, did not have any negative effect on the acceptability of the bednets by the householders. This indicates that where night time temperatures are relatively cold most of the time (as throughout most of the sub-Andean region), small mesh size bednets can be used.

L. longiflocosa has gained a greater regional importance recently because of its assumed role as the main vector in the biggest CL epidemic ever reported in Colombia, which took place between 2003 and 2004 in two municipalities in the neighbouring department of Tolima (also in the sub-Andean region), where 1,885 cases of CL were reported in 2004 alone (Pardo *et al.*, 2006). This outbreak, combined with the detection of *L. longiflocosa* in other small foci, enhances the relevance for Colombia of the results presented in this thesis, which can now be extrapolated to regions beyond Huila which share similar features: ecological, geographical, patterns of human-vector contact, ethnological, and sociological. The enhanced perception of the vectorial importance of *L. longiflocosa* also means that further studies on the epidemiology of CL in the sub-Andean region of Huila are worthwhile. In particular:

(1) Ecological studies on the sandfly vector. One of the main remaining challenges is to find natural infection with parasites (*L. braziliensis*, already found in humans) in wild *L. longiflocosa*. Future research should also focus on seasonal patterns of

L. longiflocosa abundance and its relationship with transmission rates, biting patterns of parous sandflies and survival studies. Another unexplored field concerns the identification of reservoirs.

(2) Predictive risk map of *L. longiflocosa* abundance. The regional ecological determinants of abundance for *L. longiflocosa* provide a base for generating a predictive (risk) map for this sandfly throughout Colombia (i.e. the sub-Andean area surrounding the upper and mid-Magdalena valley). This requires the incorporation into a Geographic Information System of digitized databases of altitude, temperature, rainfall and a proxy for habitat (normalized difference vegetation index) derived from satellite images. Risk map validation with sandfly data from new field surveys would be essential.

(3) Identification of local plant species which could enhance smoke effectiveness. Studies of some native plants to use as fuel for smoke to reduce sandfly biting indoors could be useful as an alternative or complementary control measure. Although the effect of smoke is immediate and short-term, the apparent concentration of transmission risk in defined periods of the year (seasons of high sandfly abundance) increases the chance that smoke could be practised during a significant proportion of the annual exposure period. If effective, this control measure could have a high probability of being sustained in the long term, as smoke is already widely practised for insect control in the endemic area (62% of householders) and is inexpensive. However, the potential negative effects of smoke on human health must also be evaluated before any recommendations are made.

(4) Adaptation of *L. longiflocosa* and *L. nuneztovari* to intensive coffee plantations. The evidence of a “partial adaptation” of *L. longiflocosa* and *L. nuneztovari* to intensive coffee plantations suggests that, because of the close location of human dwellings, there is a real possibility that these species could acquire peridomestic (synanthropic) behaviour patterns in the future and lose their apparent need for tree cover. Given the growing importance of intensive coffee plantations within the sub-Andean region, this phenomenon should be monitored, as the epidemiological consequences of further sandfly adaptation are considerable.

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**THE ECOLOGY AND CONTROL OF CUTANEOUS LEISHMANIASIS IN THE
SUB-ANDEAN REGION OF SOUTH-WEST COLOMBIA**

ANNEXES

Thesis submitted for the degree of Doctor of Philosophy in the University of London

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Annexe 1. Form to record habitat description, both general and according to K  chler's physiognomic-structural classification.

Reconocimiento Preliminar de los Fleb  tomos (  nero Lutzomyia) de la Zona de Cordillera del Departamento del Huila, Colombia

Descripci  n de habitats (Registro fitocenol  gico)

Fecha (D/M/A) _____

C  digo _____ RF- _____

C  digos de referencia en muestreos de Lutzomyia _____

Sitio _____ Vereda _____ Municipio _____

Departamento _____ Altitud (asnm) _____ Latitud " ' " Longitud " ' "

Mapa base _____ Escala _____

Tama  o del transecto ____ x ____ m

Pendiente _____ Exposici  n _____

Fotograf  a de superficie No. _____ Rollo No. _____

Paisaje (geomorfolog  a) _____

Clima (Estaci  n de ref. _____ Altitud _____ Latitud _____ Longitud _____)

Temperatura anual promedio (  C) _____ M  xima _____ M  nima _____

Precipitaci  n promedio anual (mm) _____ # d  as lluvia _____ HR (%) _____

Habitat general

☐ Bosque ☐ Caf   tradicional ☐ Caf   tecnificado al sol ☐ Caf   tecnificado semisombra

Estado sucesional y grado de intervenci  n

Sucesi  n: ☐ Primario ☐ Secundario Intervenci  n: ☐ Escasa/nula ☐ Regular ☐ Alta/muy alta

Suelo

Color (h  medo): _____ Textura: _____

Hojarasca: Composici  n _____

Cobertura (%) _____ Espesor sin descomponer ____ cm Parcial/ descompuesto ____ cm

Humedad: ☐ S   ☐ No desde ____ cm

Fauna _____

Casa muestreada m  s cercana _____ Distancia _____ m

ANALISIS ESTRUCTURAL

Seg  n K  chler (Montoya & Matos, 1967), modificado por Gabriel Paramo.

CLASES DE ALTURA (m)		FORMAS BIOL��GICAS																OTRAS EST.					
		LENOSAS						HERBACEAS			ESPECIALES												
		B	D	E	N	O	S	M	G	H	L	C	K	T	V	X	KI						
		Latifoliadas siempreverdes	Latifoliadas deciduas	Aciculares siempreverdes	Aciculares deciduas	Afilas	B+D: Semidecduas	D+E: Mixtas	Gramineas	Latifoliadas herbaceas	Liquenes, musgos	Trepadoras* (Lianas)	Tallos suculentos	Estupias	Bambues	Epifitos*	Cactiformes	Raices tabloides*	Neumatoforos*	Raices zanco*			
9	> 40																						
8	35 - 40																						
7	20 - 35																						
6	10 - 20																						
5	5 - 10																						
4	2 - 5																						
3	0.5 - 2																						
2	0.1 - 0.5																						
1	< 0.1																						

Cobertura

c: Continua (>76%)

i: Interrumpida (51-75%)

p: Manchas o grupos (26-50%)

r: Rara (6-25%)

b: Espor  dica (1-5%)

a: Casi ausente, muy rara (<1%)

Hojas

h: Duras, esclerofilas

w: Membranosas

lc: Suculentas

l: Grandes (>400cm2)

s: Peque  as (<4cm2)

N  mero de colecci  n bot  nica (###)

* Abundancia (Con relaci  n individuos le  sosos)

+++ Abundante (>50%)

++ Regular (10-49%)

+ Escaso (<10%)

Formula

1

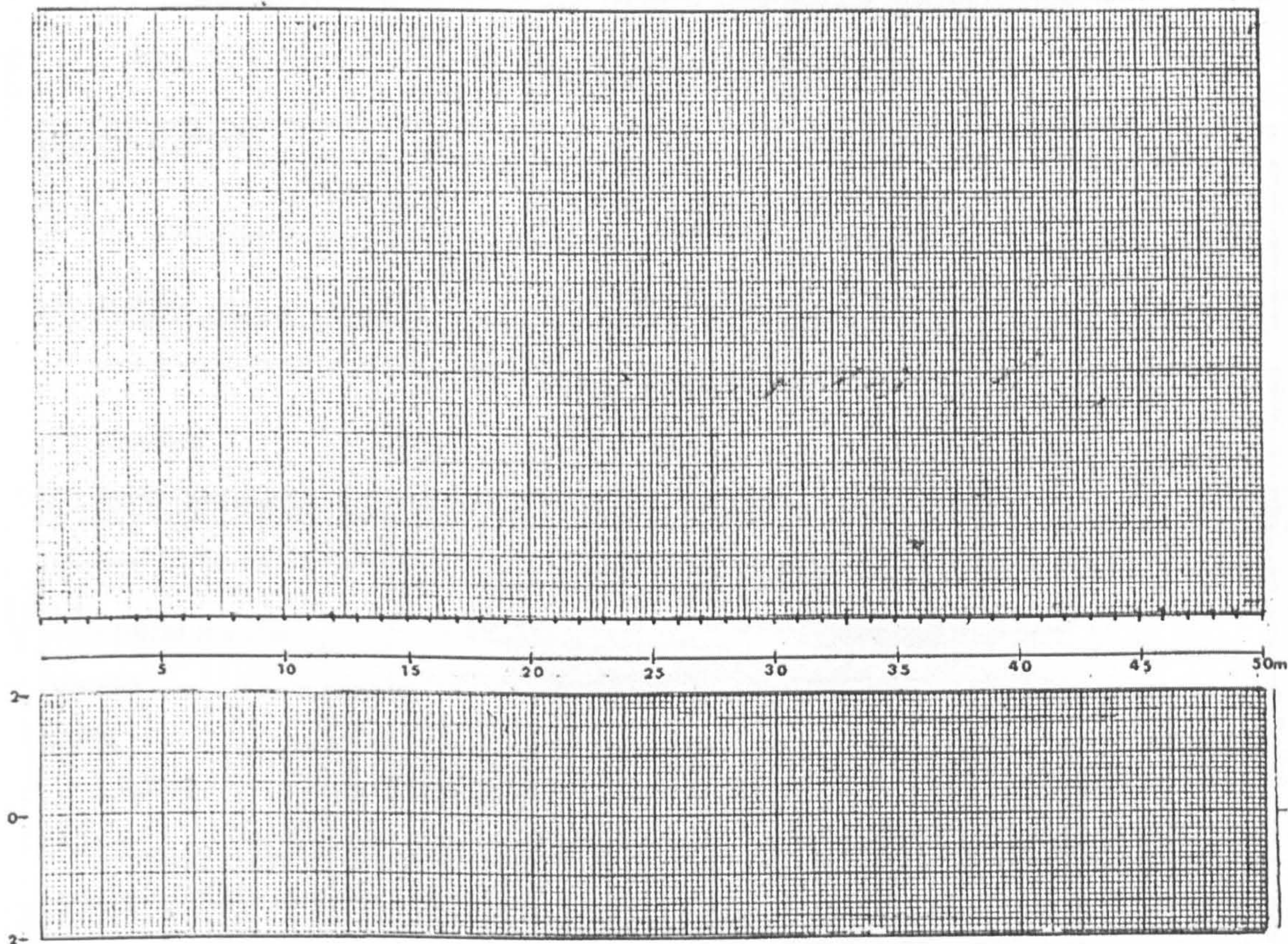
Annexe 1. Continued.

Descripción de habitats (Levantamiento de vegetación)

Levantamiento efectuado por:

Código

RF.



Especies dominantes (> cobertura, DAP> 10cm)

[illegible]

Annexe 2. Field and laboratory forms for sandfly records.

Reconocimiento preliminar del género Lutzomyia de la zona de cordillera del departamento del Huila, Colombia

Registro de especies

Fecha (D/M/A)	_____	Código	_____
Sítio	_____ Vereda _____		
Município	_____ Departamento _____		

Hábitat general		
<input type="checkbox"/> Bosque	<input type="checkbox"/> Café tecnificado al sol	<input type="checkbox"/> Pastizal
<input type="checkbox"/> Café tradicional	<input type="checkbox"/> Café tecnificado semisombra	<input type="checkbox"/> Otro _____

Condiciones climáticas					
Temperatura (°C)	_____	HR (%)	_____	Luminosidad (lux)	_____
Viento	<input type="checkbox"/> No	<input type="checkbox"/> Suave	<input type="checkbox"/> Fuerte		
Luna	<input type="checkbox"/> No	<input type="checkbox"/> Sí			
<input type="checkbox"/> Llena	<input type="checkbox"/> C. menguante	<input type="checkbox"/> Nueva	<input type="checkbox"/> C. creciente		
Lluvia	<input type="checkbox"/> No	<input type="checkbox"/> Suave	<input type="checkbox"/> Fuerte	Duración _____	

Microhábitat		
<input type="checkbox"/> Tronco árbol	<input type="checkbox"/> Herbáceo	<input type="checkbox"/> Madriguera
<input type="checkbox"/> Hojarasca	<input type="checkbox"/> Dosel	
<input type="checkbox"/> Intradomicilio (Tipo de vivienda) _____		

Método de recolección					
<input type="checkbox"/> Trampa CDC	#. _____	Altura (m)	_____	<input type="checkbox"/> Cebo humano	#. _____
<input type="checkbox"/> Trampa Chaniotis	# _____			<input type="checkbox"/> Aspiración dir. árbol	# _____

Colectores y duración del muestreo			
Hora comienzo	_____	Tiempo total	_____
Colectores	_____		No. _____
Observaciones	_____		

Captura de Lutzomyia	<input type="checkbox"/> Sí	<input type="checkbox"/> No	# insec. _____	# viales _____
----------------------	-----------------------------	-----------------------------	----------------	----------------

Registro de especies

Código _____
Fecha de procesamiento _____

Pegar rótulo
de campo
aquí

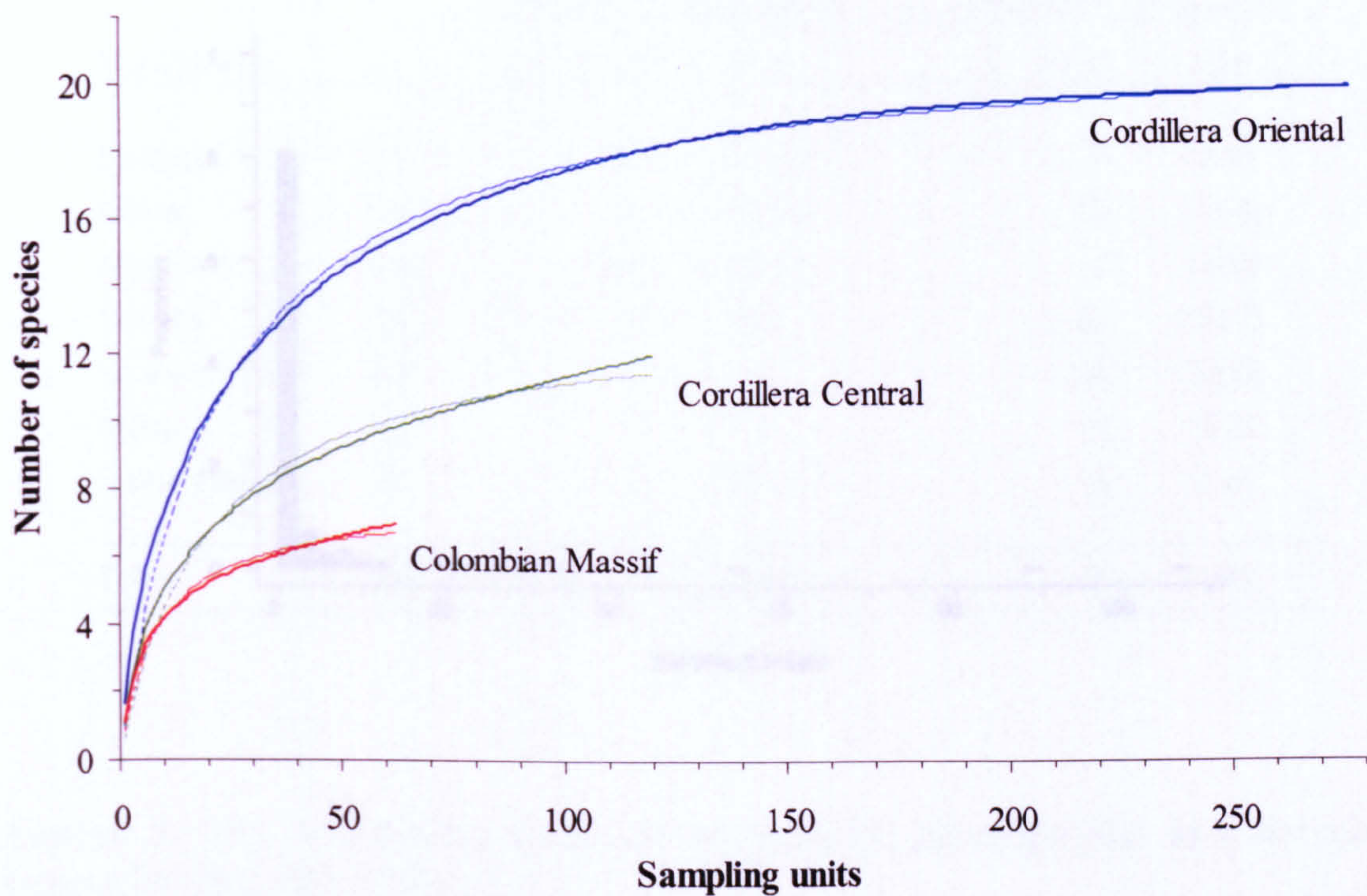
Especie	# de insectos			Observaciones
	Hembras	Machos	Total	

Annexe 3. Two ways ordered table generated by TWINSpan based on physiognomic-structural features of 57 sampled sites. The classification of sites in groups (highlight by the same colour) is indicated along the bottom margin (See section 2.2.5 for explanation). Sites 2, 9, and 30 were regrouped based on the presence/absence of similar tree strata (cover $\geq 6\%$).

[illegible]

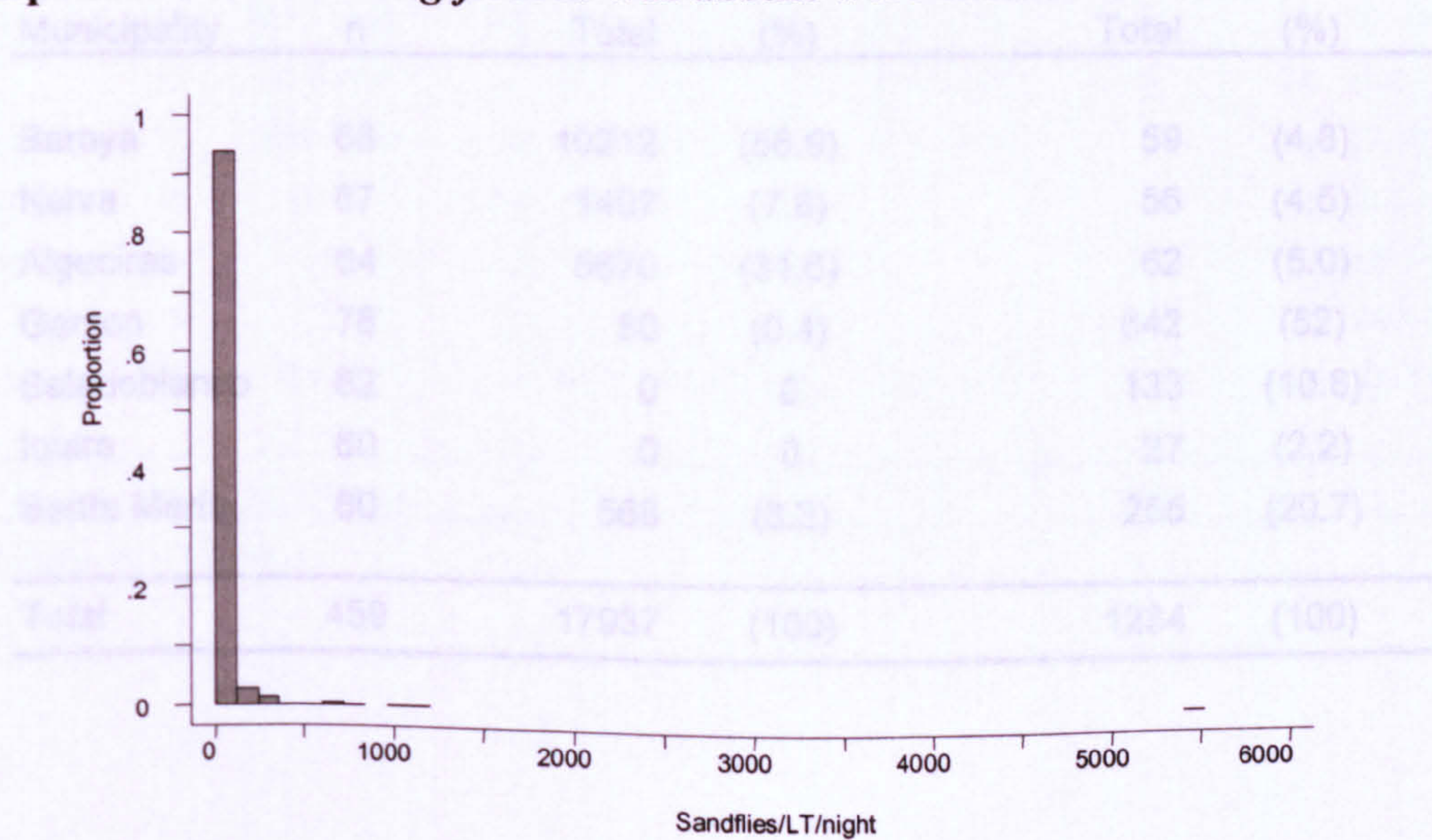
^a See Table 2.2 for explanation; ^b Indicates that leaf phenology was not determined, except for coffee plantations sites where plants were coffee, classified as evergreen (B3 and B4). Values within the table indicates a scale of abundance (cover %): 1 = < 1%; 2 = 1 - 5%; 3 = 6 - 25%; 4 = 26 - 50%; 5 = 51 - 75%; 6 = ≥76%; - = species absent.

Annexe 4. Observed and fitted species accumulation curves by region according to catches with CDC traps outdoors. Solid lines represent observed data and dashed lines show the predictions of the Clench model.

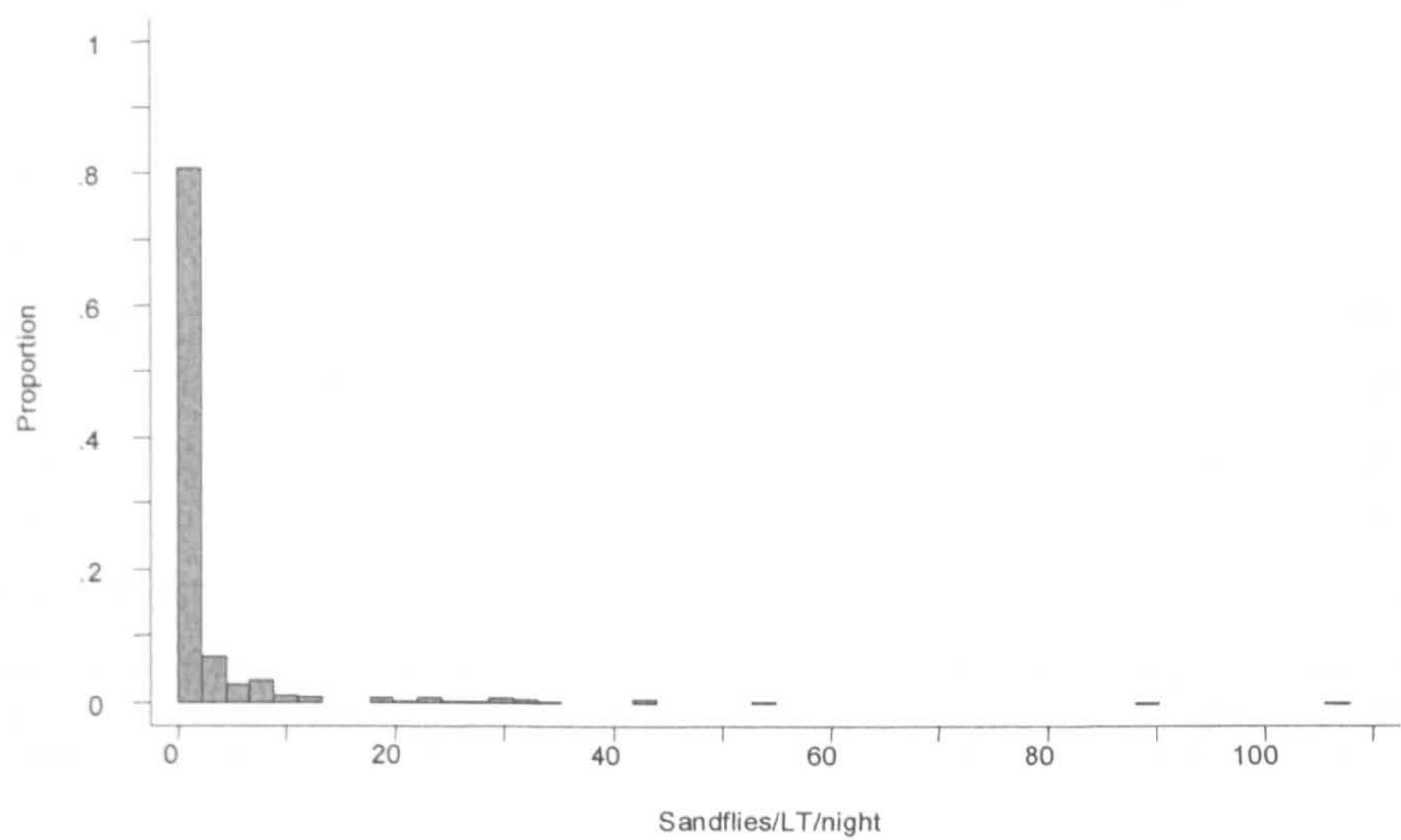


Annexe 7. The relationship between is biodiversity and the total number of sandflies collected in CDC light traps outdoors.

Annexe 5. Frequency distribution of the raw data of *L. longiflocosa* (n = 337, total sandflies = 17,937) according to outdoor CDC light traps. Data from two municipalities where *L. longiflocosa* was absent were excluded.



Annexe 6. Frequency distribution of the raw data of *L. nuneztovari* (n = 459, total sandflies = 1,233) according to outdoor CDC light traps.



Annexe 7. The relationship between municipality and the total number of sandflies collected in CDC light traps outdoors.

Municipality	n	<i>Lutomyia longiflocosa</i>		<i>Lutzomyia nuneztovari</i>	
		Total	(%)	Total	(%)
Baraya	68	10212	(56.9)	59	(4.8)
Neiva	67	1407	(7.8)	56	(4.5)
Algeciras	64	5670	(31.6)	62	(5.0)
Garzon	78	80	(0.4)	642	(52)
Saladoblanco	62	0	0	133	(10.8)
Iquira	60	0	0	27	(2.2)
Santa María	60	568	(3.2)	255	(20.7)
Total	459	17937	(100)	1234	(100)

Annexe 8. The relationship between municipality and the total number of sandflies collected in CDC light traps indoors.

Municipality	n	<i>Lutzomyia longiflocosa</i>		<i>Lutzomyia nuneztovari</i>	
		Total	(%)	Total	(%)
Baraya	12	165	(22.1)	7	(7.9)
Neiva	19	129	(17.3)	12	(13.5)
Algeciras	14	436	(58.4)	4	(4.5)
Garzon	18	0	0.0	30	(33.7)
Saladoblanco	17	0	0	29	(32.6)
Iquira	15	0	0	4	(4.5)
Santa María	13	16	(2.1)	3	(3.4)
Total	108	746	(100)	89	(100)

Annexe 9. The relationship between the sampled municipalities and the mean human landing rate outdoors.

Municipality	n	<i>Lutzomyia longiflocosa</i>			<i>Lutzomyia nuneztovari</i>		
		Total female/male	GM sandflies/person/40 min		Total females ^a	GM sandflies/person/40 min	
			Female (95% C.I.)	Male (95% C.I.)		Female (95% C.I.)	
Baraya	17	4175 / 359	23 (4.7 - 104)	3 (0.5 - 9.3)	8	0.3 (0 - 0.6)	
Neiva	20	1018 / 94	17 (6.7 - 39)	1.4 (0.3 - 3.3)	24	0.6 (0.2 - 1.3)	
Algeciras	17	2609 / 679	13 (3.0 - 49)	1.9 (0.1 - 6.7)	2	0.1 (0 - 0.2)	
Garzon	18	18 / 0	0.4 (0 - 0.9)	0	44	1.1 (0.4 - 2.4)	
Saladoblanco	11	0	0	0	29	0.7 (-0.2 - 2.6)	
Iquira	8	0	0	0	7	0.5 (-0.1 - 1.6)	
Santa María	12	54 / 7	1 (-0.1 - 3.3)	0.3 (-0.1 - 0.9)	16	0.6 (0 - 1.6)	
total	103	7874 / 1139			130		

^a Males were not collected.

Annexe 10. The relationship between the sampled municipalities and the abundance of sandflies resting on tree trunks.

<i>Lutzomyia longiflocosa</i>					<i>Lutzomyia nuneztovari</i>				
		GM sandflies/person/40 min					GM sandflies/person/40 min		
Municipality	n	Total female/male	Female (95% C.I.)	Male (95% C.I.)	Total female/male	Female (95% C.I.)	Male (95% C.I.)		
Baraya	8	436 / 52	12 (1.4 - 75)	0.9 (-0.4 - 5.3)	1 / 0	0.1 (-0.1 - 0.3)	0		
Neiva	8	14 / 39	1.2 (0.2 - 3.1)	2.3 (0.2 - 7.8)	0 / 2	0	0.2 (-0.2 - 0.6)		
Algeciras	6	23 / 3	2.1 (0 - 8.2)	0.4 (-0.2 - 1.2)	0	0	0		
Garzon	7	1 / 3	0.1 (-0.1 - 0.4)	0.2 (-0.3 - 1.0)	1 / 0	0.1 (-0.1 - 0.4)	0		
Saladoblanco	8	0	0	0	0 / 2	0	0.2 (-0.2 - 0.6)		
Iquira	4	0	0	0	0	0	0		
Santa María	4	0	0	0	1 / 6	0.2 (-0.3 - 1.1)	0.6 (-0.7 - 6.7)		
Total	45	474 / 97			3 / 10				

Annexe 11. The relationship between general habitat type and indoor sandfly abundance (as measured by CDC light traps).

<i>Lutzomyia longiflocosa</i> ^a					<i>Lutzomyia nuneztovari</i>				
		GM sandflies/trap/night (95% C.I.)					GM sandflies/trap/night (95% C.I.)		
Habitat type	n	Female	Male	Total	n	Female	Male	Total	
forest	20	1.4 (0.4 - 3.0) ^a	0.2 (0 - 0.5)	1.7 (0.6 - 3.4)	32	0.6 (0.2 - 1.1)	0.1 (0 - 0.2)	0.6 (0.2 - 1.2)	
traditional coffee	17	3.9 (0.9 - 11)	0.3 (0 - 0.7)	4.3 (1.1 - 12)	19	0.2 (0 - 0.5)	0.04 (0 - 0.1)	0.2 (0 - 0.6)	
intensive semishaded coffee	14	0.1 (0 - 0.4)	0	0.1 (-0.1 - 0.4)	22	0.2 (0 - 0.4)	0	0.2 (0 - 0.4)	
intensive unshaded coffee	25	2.7 (1.0 - 5.5)	0.5 (0.2 - 0.9)	3.1 (1.3 - 6.3)	35	0.2 (0 - 0.4)	0.1 (0 - 0.2)	0.3 (0.1 - 0.6)	
total	76	1.8 (1.0 - 2.8)	0.3 (0.2 - 0.4)	2.0 (1.2 - 3.2)	108	0.3 (0.2 - 0.5)	0.06 (0 - 0.1)	0.4 (0.2 - 0.5)	
(total sandflies)		(711)	(35)	(746)		(78)	(11)	(89)	

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent.

Annexe 12. The relationship between general habitat type and the mean human landing rate outdoors.

Habitat type	<i>L. longiflocosa</i> ^a				<i>L. nuneztovari</i>		
	n	GM			n	GM	
		sandflies/person/40 min (95% C.I.)				sandflies/person/40 min (95% C.I.)	
		Females	Males	Total		Females	Males
forest	37	11 (4.5 - 26)	1.8 (0.6 - 3.9)	12 (4.8 - 28)	48	0.8 (0.4 - 1.3)	0
traditional coffee	18	7.2 (1.5 - 26)	1.0 (0 - 3.3)	7.6 (1.6 - 28)	19	0.3 (0 - 0.6)	0
intensive semishaded coffee	11	1.6 (-0.3 - 2.7)	0	0.6 (-0.3 - 2.7)	14	0.5 (0 - 1.3)	0
intensive unshaded coffee	18	5.8 (1.2 - 12)	0.9 (0.1 - 2.1)	5.9 (1.3 - 21)	22	0.2 (0 - 0.6)	0
total	84	6.6 (3.6 - 11)	1.1 (0.5 - 1.9)	6.9 (3.8 - 12)	103	0.5 (0.3 - 0.8)	0
(total sandflies)		(7874)	(1139)	(9013)		(130)	0

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent.

Annexe 13. The relationship between general habitat type and sandfly abundance on tree trunk resting sites.

Habitat type	<i>L. longiflocosa</i> ^a				<i>L. nuneztovari</i>			
	n	GM sandflies/person/40min			n	GM sandflies/person/40min		
		Females	Males	Total		Females	Males	Total
forest	21	2.8 (0.7 - 7.4)	0.7 (0.1 - 1.9)	3.8 (1.2 -9.7)	29	0.05 (0 - 0.1)	0.04 (0 - 0.1)	0.1 (0 - 0.2)
traditional coffee	9	1.0 (0.2 - 2.6)	1.0 (0 - 3.1)	1.8 (0.2 - 5.4)	10	0.1 (-0.1 - 0.2)	0.4 (-0.2 - 1.2)	0.5 (-0.1 - 1.4)
intensive semishaded coffee	3	0	0	0	6	0	0	0
total	33	1.9 (0.7 - 3.8)	0.7 (0.2 - 1.5)	2.6 (1.1 - 5.3)	45	0.05 (0 - 0.1)	0.1 (0 - 0.2)	0.1 (0 - 0.3)
(total sandflies)		(474)	(97)	(571)		(3)	(10)	(13)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent.

Annexe 14. Plant species (mainly plants with diameter to the breast height > 10 cm) found in the Sub-Andean region of Huila department.

Scientific name	Common name	Type of foliation ^a	Municipality of collection ^b	General habitat ^c
Anacardiaceae				
<i>Mangifera indica</i> L. ^d	Mango	dec	IQ, SA, GA	tra, ses
<i>Mauria heterophylla</i> Kunth	Caspicaracho	dec	AL	for
<i>Mauria suaveolens</i> Poepping	Chuncho	dec	BA	for
cf. <i>Tetragastris</i> sp.	Cedrillo	sem	IQ	uns
<i>Toxicodendron striatum</i> (R & P.) ^d	Caspicaracho o Pedrohemandez	sem	NE	for
Annonaceae				
<i>Xylopia</i> sp.	Laurel blanco	dec	NE	for
<i>Annona muricata</i> L. ^d	Guanabana	sem	GA	ses
Aquifoliaceae				
cf. <i>Ilex</i> sp.	Surumbo	eve	AL	for
Araliaceae				
<i>Dendropanax arboreus</i>		dec	AL	for
<i>Oreopanax cecropifolium</i> Cuatr.	Papayuelo o Flautón	sem	AL, NE	for
Arecaceae				
<i>Alphanea</i> sp. ^d	Palma de chonta	eve	IQ	for, uns
cf. <i>Iriartea</i> sp.	Palma Bombona	eve	SA	for
<i>Phytelephas</i> sp.	Palma cunaja o Cabeza de negro	eve	GA	for
<i>Prestoea</i> sp.	Palmo	eve	IQ	for
sp.	Palma San Pablo	eve	AL	for
Asteraceae				
<i>Montanoa quadrangularis</i>	Arbol loco	eve	GA, BA	for
<i>Pollalesta discolor</i> (H. B. K.) Aristeguieta	Congo	sem	IQ	ses
sp.			NE	for
Bombacaceae				
<i>Ochroma tomentosa</i> Willd.	Balso	dec	AL, NE	for
Boraginaceae				
<i>Cordia alliodora</i> (R. & P.) Oken	Mo, Moncuro o Nogal	dec	AL, NE, BA	tra
Burseraceae				
<i>Protium</i> sp.		dec	IQ	for
Buxaceae				
<i>Styloceras laurifolium</i> (Willd.) H.B.K.		dec	NE	for
Caesalpinaceae				
<i>Cassia</i> sp.	Vainillo o frijolito	dec	GA, AL, NE, BA	tra, ses
Caprifoliaceae				
<i>Viburnum cornifolium</i> Killip & Smith	Cabo de hacha o maiz tostado	sem	SM, AL, NE, BA	for
Caricaceae				
<i>Carica papaya</i> ^d	Papaya	eve	SA, GA	ses
<i>Carica</i> sp.	Papayuelo	eve	NE	for
Cecropiaceae				
<i>Cecropia</i> spp. ^d	Yarumo, Guarumo	eve	IQ, GA, AL, BA	for, ses, uns
Chloranthaceae				
<i>Hedyosmum</i> cf. <i>racemosum</i> (R. & P.) G. Don.	Granizo	dec	SA	for
Chrysobalanaceae				
<i>Hirtella</i> cf. <i>americana</i> L.		eve	GA	for
Clusiaceae				
<i>Clusia</i> cf. <i>amazónica</i> Pl. & Tr.	Cope	dec	AL	for
<i>Clusia</i> cf. <i>discolor</i> Cuatr.	Cope	dec	SA	for
<i>Clusia</i> sp. 1	Cope	dec	SA, GA	for
<i>Clusia</i> sp. 2	Caucho	dec	BA	for
<i>Clusia</i> sp. 3	Cope	dec	SM, AL	for
<i>Clusia</i> sp.	Cope	dec	SA	for

Annexe 14. Continued.

Scientific name	Common name	Type of foliation ^a	Municipality of collection ^b	General habitat ^c
<u>Cochlospermaceae</u>				
<i>Cochlospermum</i> sp.	Oreja de Mula	dec	SA, NE	for
<u>Cunoniaceae</u>				
<i>Weinmannia pubescens</i>	Encenillo	sem	AL	for
<u>Cyatheaceae</u>				
sp. ^d	Helecho arborescente, Palma boba	eve	IQ, SA, GA	for
<u>Elaeocarpaceae</u>				
<i>Mutingia calabura</i> L.	Huesito	dec	BA	for
<u>Ericaceae</u>				
<i>Cavendishia bracteata</i> (R. & P. ex J. St. Hill)	Ubillo	eve	AL	for
<u>Escalloniaceae</u>				
<i>Escallonia paniculata</i> (R. & P.) Roem. & Schult	Punta de lanza	eve	BA	for
<u>Euphorbiaceae</u>				
<i>Alchomea</i> cf. <i>glandulosa</i> Endl. & Poepp.	Arrayan	dec	SA, NE	for
<i>Alchomea</i> sp.	Clavo pasado	dec	GA, NE	for
<i>Hyeronima</i> sp.		dec	SA, AL	for
<i>Mabea</i> sp.	Canilla de pisco	eve	GA	for
<i>Tetrorchidium</i> cf. <i>boyacanum</i> Croiz.		eve	AL	for
<u>Fagaceae</u>				
<i>Quercus humboldtii</i> Bonpl.	Roble, Roble blanco	dec	SA, AL, NE, BA	for
<u>Flacourtiaceae</u>				
<i>Casearia mollis</i> H.B.K.	Mono o caspicaracho	dec	SA, NE, BA	for, uns
<i>Casearia arborea</i>	Café de montaña	dec	GA	for
<i>Hasseltia floribunda</i> H.B.K.		eve	AL	for
<u>Hipocastanaceae</u>				
<i>Billia columbiana</i> (H. B. K.) Pl.	Diomate	dec	BA	for
<u>Hypericaceae</u>				
<i>Vismia baccifera</i> (L.) Tr. & Pl.	Lacre	sem	GA	for
<u>Lauraceae</u>				
<i>Aniba puchury-minor</i> (Mart.) Mez.	Laurel	eve	GA	for
<i>Aniba</i> sp.	Laurel	eve	BA	for
<i>Cinnamomum cinnamomifolium</i> (H. B. K.) Kosterm	Arrayan, Cucharo	eve	NE, BA	for
<i>Nectandra acutifolia</i> Mez.	Aguacatillo, Laural mierda, Café	sem	SA, NE	for
<i>Nectandra lineata</i> (H. B. K.) Rohwer		sem	SM	tra
<i>Nectandra longifolia</i> (R. et P.) Ness	Laurel	eve	NE	for
<i>Ocotea</i> cf. <i>puberula</i> (Rich.) Nees	Laurel comino	eve	BA	for
<i>Ocotea macrophylla</i> Kunth in H. B. K.	Laurel de peña	eve	BA	for
<i>Persea americana</i> Miller	Aguacate	dec	SM, SA, AL, NE, BA	tra, ses, uns
<i>Persea</i> cf. <i>caerulea</i> (Ruiz & Pav.) Mez.		eve	SM, IQ	for
sp.	Rapabarbo		NE	for
<u>Magnoliaceae</u>				
cf. <i>Dugandiodendron</i> sp.	Cobre	dec	AL	for
<u>Melastomataceae</u>				
<i>Miconia longifolia</i> (Aubl.) DC.	Garruncho	eve	SA, NE	for
<i>Miconia reducens</i> Tr.		eve	IQ	for
<i>Miconia trinervia</i> (Sw.) D. Don ex Laud.		eve	IQ	for
<u>Meliaceae</u>				
<i>Cedrela</i> cf. <i>angustifolia</i> Sesse & Moc		dec	NE	for
cf. <i>Cedrela odorata</i>	Cedro rosado	dec	NE	tra
<i>Cedrela</i> sp.	Cedro rosado	dec	NE	for
<u>Mimosaceae</u>				
<i>Acacia</i> sp. 1	Acacia	dec	GA	ses
cf. <i>Acacia</i> sp. 2	Muche	dec	BA	tra

Annexe 14. Continued.

Scientific name	Common name	Type of foliation ^a	Municipality of collection ^b	General habitat ^c
<i>Inga aff. edulis</i>	Guamo; Guamo blanco	sem	GA	tra
<i>Inga aff. Macrophylla</i>	Guamo	sem	BA	for, tra
<i>Inga codonantha</i> Pittier	Guamo	sem	SM	ses
<i>Inga culagana</i> Britton et Killip	Guamo, Guamo cerindo	sem	AL, NE	for, tra
<i>Inga macrophylla</i> Humb. & Bonpl. ex Willd.	Guamo, Guamo cerindo	sem	NE, BA	for, tra
<i>Inga punctata</i> Willd.	Cerindo, Vainillo	sem	GA	for
<i>Inga</i> sp. 1	Guamo Cerindo	sem	SA	for
<i>Inga</i> sp. 2	Guamo Cerindo	sem	AL	tra
<i>Inga</i> sp. 3		sem	NE	for
<i>Inga</i> sp. 4	Guamo Cerindo	sem	BA	for
<i>Inga</i> spp.	Guamo	sem	IQ, SA, GA, AL , BA	for, tra, ses, uns
Moraceae				
<i>Ficus cf. andicola</i> Standley	Higueron; Caucho blanco	dec	SM, GA	for
<i>Ficus insipida</i> Willd.		dec	SM, IQ	for, tra
<i>Ficus</i> sp. 1		dec	AL	for
<i>Ficus</i> sp. 2	Caucho blanco	dec	NE	for
<i>Ficus</i> sp. 3	Caucho higueron	dec	BA	for
<i>Ficus</i> sp. 4		dec	SM	for
<i>Ficus</i> sp. 5		dec	AL	for
<i>Maciura tinctoria</i> (L.) Steud		dec	IQ	for
sp.	Palo Blanco		GA	for
<i>Trophis caucana</i> (Pittier) C.C. Bery.	Lechoso, Leche de chiva, Arenillo	dec	SM, SA, GA, NE	for
<i>Trophis</i> sp.	Caucho	dec	BA	for
Musaceae				
<i>Musa</i> spp. ^d	Banano, platano	eve	SM, IQ, SA, GA AL, NE, BA	tra, ses, uns
<i>Heliconia</i> spp. ^d		eve	IQ	for
Myrsinaceae				
<i>Myrsine latifolia</i> (R. & P.) Spreng.	Garruncho colorado	eve	SA	for
<i>Stylogyne</i> sp.	Cucharo	eve	AL	for
Myrtaceae				
<i>Eugenia jambos</i> L.	Pomamoso	eve	BA	tra
<i>Eugenia</i> sp.		eve	SM	for
<i>Myrcia cf. popayanensis</i>	Arrayan	eve	BA	for
<i>Myrcia</i> sp. 1		eve	GA	for
<i>Myrcia</i> sp. 2		eve	IQ	for
<i>Myrciantes cf. rhopaloides</i> (H. B. K.) Mc. Vaugh	Garruncho escobo	eve	SA	for
<i>Psidium guajava</i> L. ^d	Guayaba	dec	SA, GA, BA	tra, ses, uns
Papilionaceae				
<i>Erythrina edulis</i> Triana ex Micheli	Chachafuto	dec	GA	ses
<i>Erythrina</i> sp.	Cachingo, Cambulo	dec	SM, SA,GA AL, NE, BA	for, tra, ses, uns
<i>Gliricidia sepium</i> (Jacq.) Steud	Matarraton	dec	IQ, GA	ses
Piperaceae				
<i>Piper</i> sp.		eve	NE	for
Poaceae				
<i>Bambusa guadua</i> H. et B. ^d	Guadua	eve	SM, IQ, AL	for, tra
Rosaceae				
<i>Prunus</i> sp.		eve	AL	for
Rubiaceae				
<i>Chomelia barbellata</i> Standl.		dec	IQ	for
<i>Coffea arabica</i> L. var. colombia ^d	café colombia	eve	SM, GA, NE, BA	ses, uns
<i>Coffea arabica</i> L. var. caturra ^d	café caturra	eve	SM, IQ, SA, GA AL, NE, BA	tra, ses, uns

Annexe 14. Continued.

Scientific name	Common name	Type of foliation ^a	Municipality of collection ^b	General habitat ^c
<i>Coffea arabica</i> L. var. <i>typica</i> ^d	café común	eve	SM, IQ, GA, AL NE, BA	tra, ses, uns
<i>Diocodendron dioicum</i>		dec	AL	for
<i>Hamelia cf. patens</i> Jacq.		eve	BA	tra
<i>Ladenbergia</i> sp.	Hojiancho	eve	SA	for
<i>Palicourea cf. demissa</i> Standl.		eve	NE	for
<i>Palicourea cf. thyrsiflora</i>	Chilco	eve	GA	for
<i>Palicourea demissa</i> Standl.		eve	GA	for
<i>Posoqueria cf. panamensis</i> Walp.	Guacharaco	eve	NE	for
sp.	Canoa		GA	for
Rutaceae				
<i>Citrus sinensis</i>	Naranjo	dec	SA, GA, BA	tra, ses, uns
<i>Zanthoxylum</i> sp.		dec	AL	for
Sapindaceae				
<i>Cupania americana</i> L.	Guacharaco, Guamo de monte	dec	SM, AL, BA	for, tra
<i>Melicocca bijuga</i> ^d	Mamoncillo	dec	GA	ses
Simaroubaceae				
<i>Simarouba amara</i> Aubl.		dec	AL	for
Solanaceae				
<i>Solanum</i> sp. 1		eve	BA	for
<i>Solanum</i> sp. 2	Trapiche	eve	GA	for
Sterculiaceae				
<i>Theobroma cacao</i> L. ^d	Cacao	eve	SM, IQ, SA, GA, NE	tra, ses
Symplocaceae				
<i>Symplocos mucronata</i>	Cafesillo	eve	SA	for
Theaceae				
<i>Freziera</i> sp.	Monday	dec	GA	for
Tiliaceae				
<i>Heliocharpus cf. americana</i> (h. B. K.) Meizer	Cadillo	dec	IQ	for
Ulmaceae				
<i>Trema micrantha</i> (L.) Blume	Surrumbo	dec	GA	for
Urticaceae				
<i>Urera cf. baccifera</i> (L.) Gaud.	Pringamoso silvestre	sem	IQ	for
<i>Urera cf. elata</i> (Sw.) Griseb.	Pringamoso	sem	NE, BA	for
Verbenaceae				
<i>Duranta coriacea</i>	Totoco, Cruceto	eve	AL, NE	for
<hr/>				
Total species: 135	Total genera: 91	Total families: 53		

^a eve: evergreen, dec: deciduous, sem: semideciduous; ^b SM: Santa María, IQ: Iquira, SA: Saladoblanco, GA: Garzon, AL: Algeciras, NE: Neiva, BA: Baraya; ^c Habitat where the species was collected; for: forest, tra: traditional coffee, ses: intensive semishaded coffee, uns: intensive unshaded coffee; ^d Well known species identified in situ by common name.

Annexe 15. The relationship between the presence of plant families, detected in at least six of 62 sampled sites (five sites were excluded because of poor plant identification), and sandfly abundance (as measure by outdoor CDC light traps).

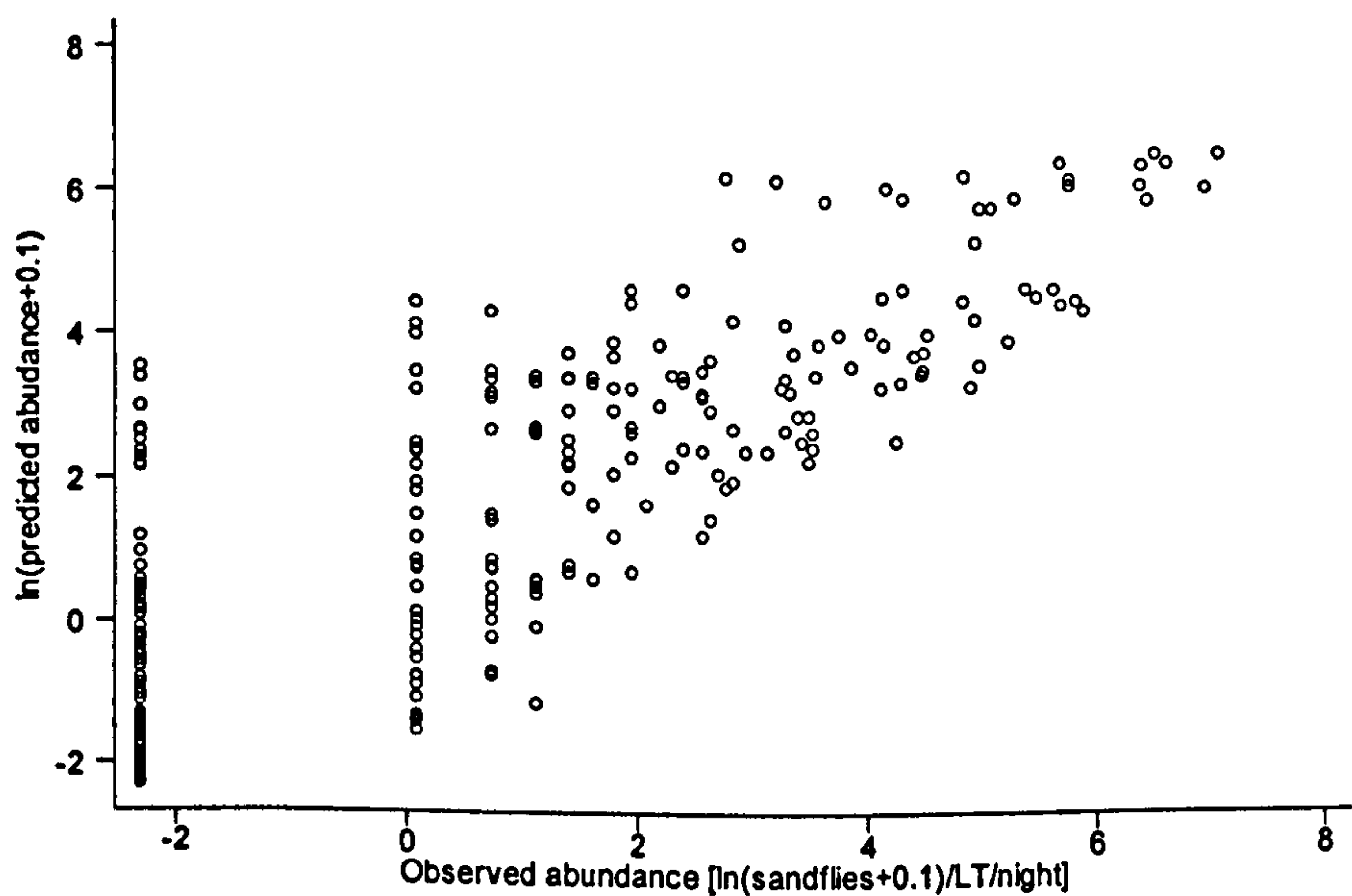
Family	<i>Lutzomyia longiflocosa</i> ^a			<i>Lutzomyia nuneztovari</i>		
	n	GM	(95% C.I.)	n	GM	(95% C.I.)
Arecaceae^b						
presence	36	1.5	(0.7 - 2.8)	72	1.1	(0.6-1.7)
absence	115	8.3	(5.3 - 13)	143	1.1	(0.8-1.5)
Clusiaceae^b						
presence	43	10	(4.9-21)	67	1.4	(0.8-2.2)
absence	92	5.1	(3.1-8.1)	112	0.9	(0.5-1.2)
Cyatheaceae^b						
presence	16	2.6	(1.2 - 5.0)	44	1.7	(0.8-3)
absence	135	6.3	(4.2 - 9.4)	171	1.0	(0.7-1.3)
Fagaceae^b (<i>Quercus humboldtii</i>)						
presence	44	6.9	(3.7-12)	52	0.3	(0.1-0.4)
absence	91	6.2	(3.6-10)	127	1.5	(1-2.1)
Lauraceae^c						
presence	112	6.6	(4-10)	128	0.6	(0.4-0.8)
absence	103	4.6	(2.9-6.9)	139	1.2	(0.8-1.6)
Mimosaceae^c (mainly <i>Inga</i> spp.)						
presence	96	4.4	(2.4-7.5)	104	0.6	(0.4-0.9)
absence	119	6.6	(4.5-9.6)	163	1.1	(0.8-1.4)
Moraceae^c						
presence	67	15	(8.5-26)	103	1.2	(0.8-1.7)
absence	148	3.3	(2.2-4.8)	164	0.7	(0.5-1)
Musaceae^d (<i>Musa</i> spp.)						
presence	155	1.4	(0.9-2)	166	0.6	(0.4-0.8)
absence	31	9.0	(3.4-22)	78	0.6	(0.4-0.8)
Myrtaceae^c						
presence	32	11	(5.4-21)	48	3.3	(1.9-5.3)
absence	183	4.9	(3.4-7)	219	0.6	(0.4-0.7)
Papilionaceae^c (<i>Erythrina</i> spp.)						
presence	60	1.3	(0.6-2.3)	68	0.6	(0.3-0.9)
absence	155	8.8	(6-13)	199	1.0	(0.7-1.3)
Rubiaceae^c						
presence	36	13	(6-26)	52	1.7	(0.9-2.8)
absence	179	4.6	(3.2-6.6)	215	0.7	(0.5-0.9)

^a GM values exclude data from the two municipalities where *L. longiflocosa* was absent; ^b Only forest sites were included; ^c Only forest and traditional cofee sites were included; ^d Only coffee plantations were included.

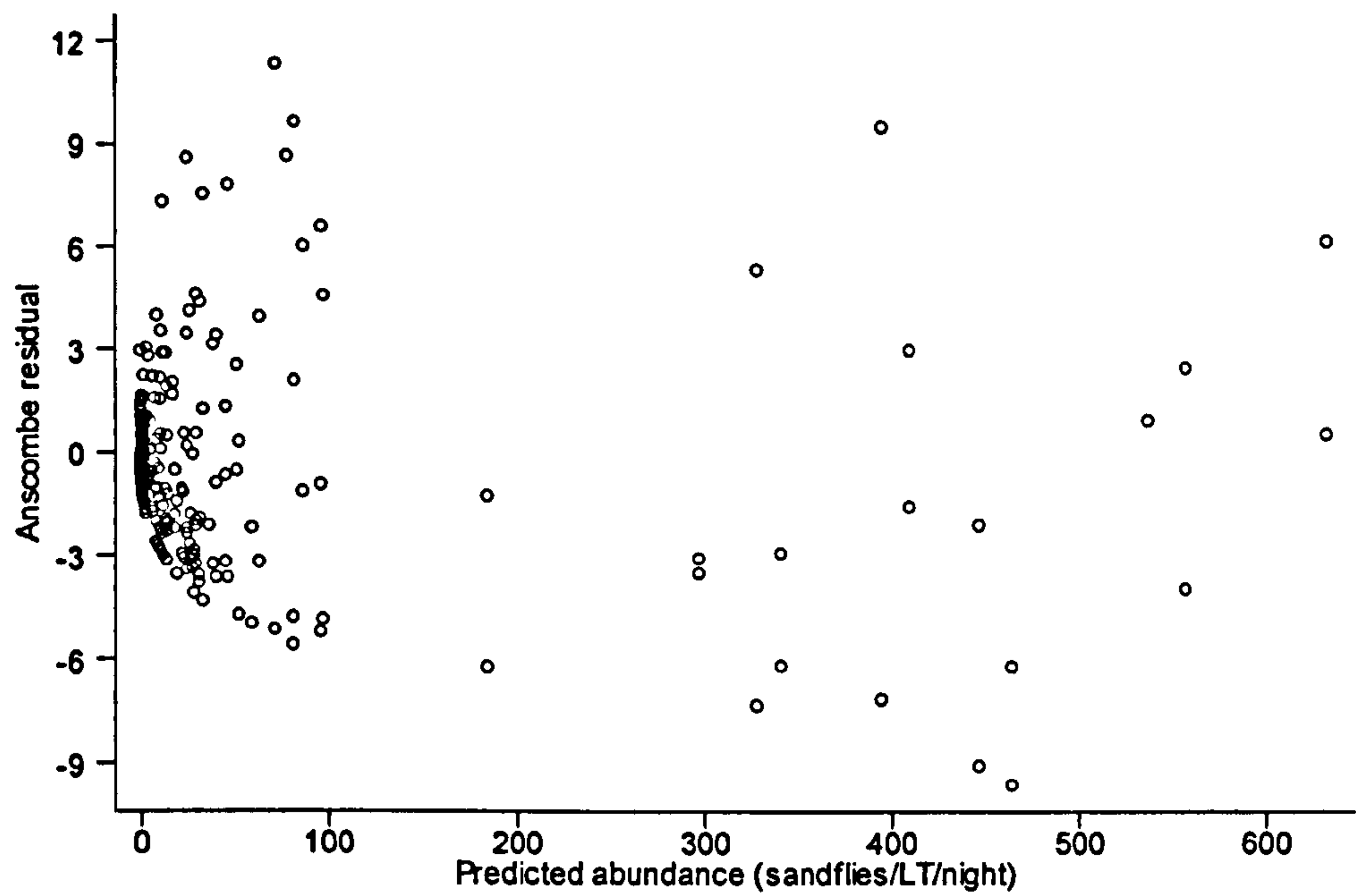
Annexe 16. Significance of ecological determinants for *Lutzomyia longiflocosa* abundance identified by multivariate analysis (MAM with assumption of Negative binomial errors).

Explanatory variable	χ^2	df	p
Local determinants			
General habitat	42.59	2	<0.001
Slope in the sampling site	181.99	7	<0.001
Degree of protection from wind	23.45	2	<0.001
Number of tree strata	69.63	4	<0.001
Cover	39.53	1	<0.001
Litter cover	25.00	3	<0.001
Regional determinants			
Altitude	55.24	1	<0.001
Altitude square	47.03	1	<0.001
Rainfall	5.03	1	0.025
Rainfall square	6.02	1	0.014
Temperature	9.05	1	0.003
Temperature square	8.86	1	0.003
Slope	122.69	3	<0.001
Soil type	236.51	7	<0.001

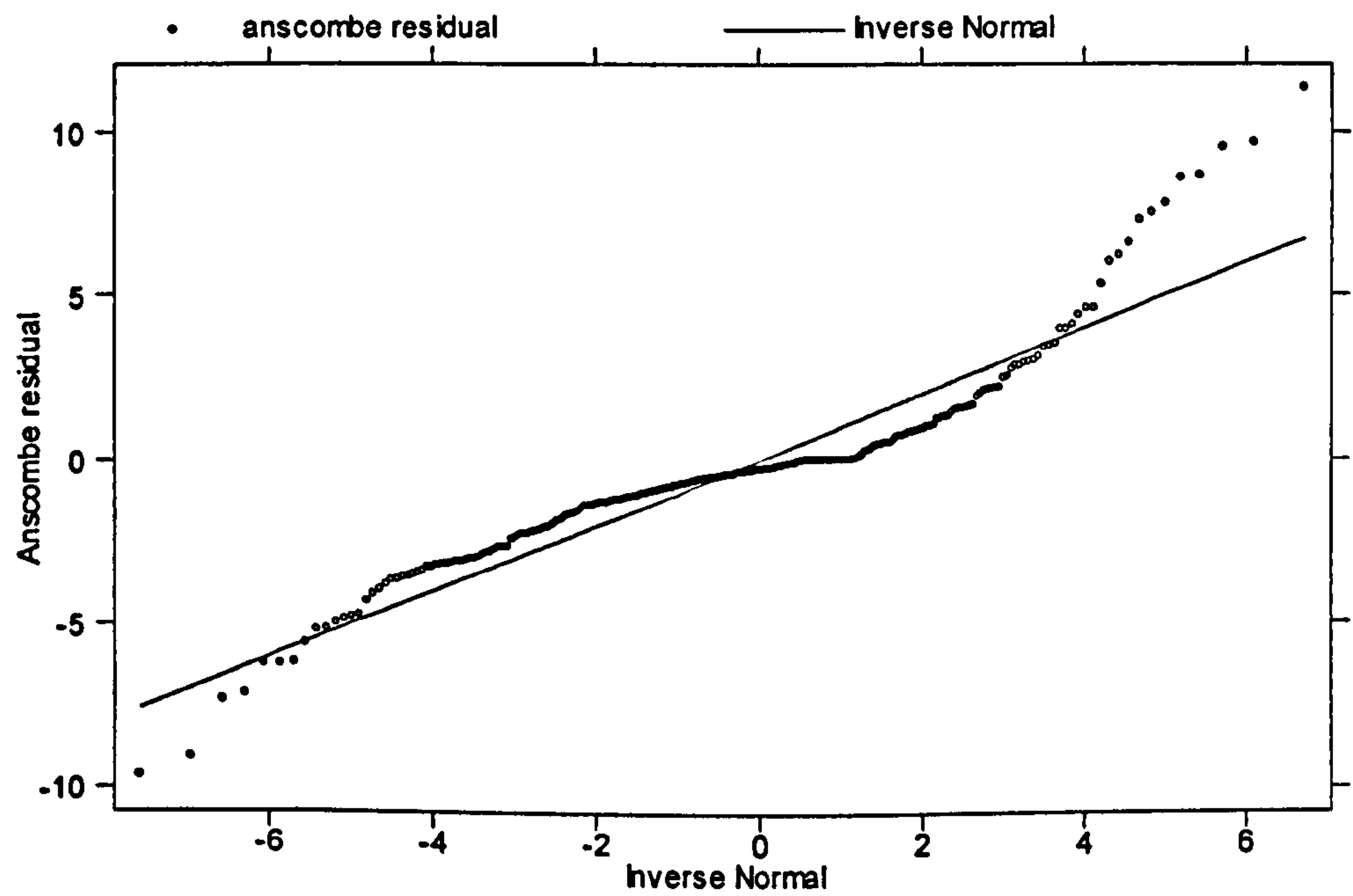
Annexe 17. Observed log transformed outdoor abundance of *Lutzomyia longiflocosa* (collected with CDC light traps) against their predicted value according to the MAM incorporating thirteen explanatory variables.



Annexe 18. Raw outdoor abundance of *Lutzomyia longiflocosa* predicted by the MAM against their Anscombe residuals.



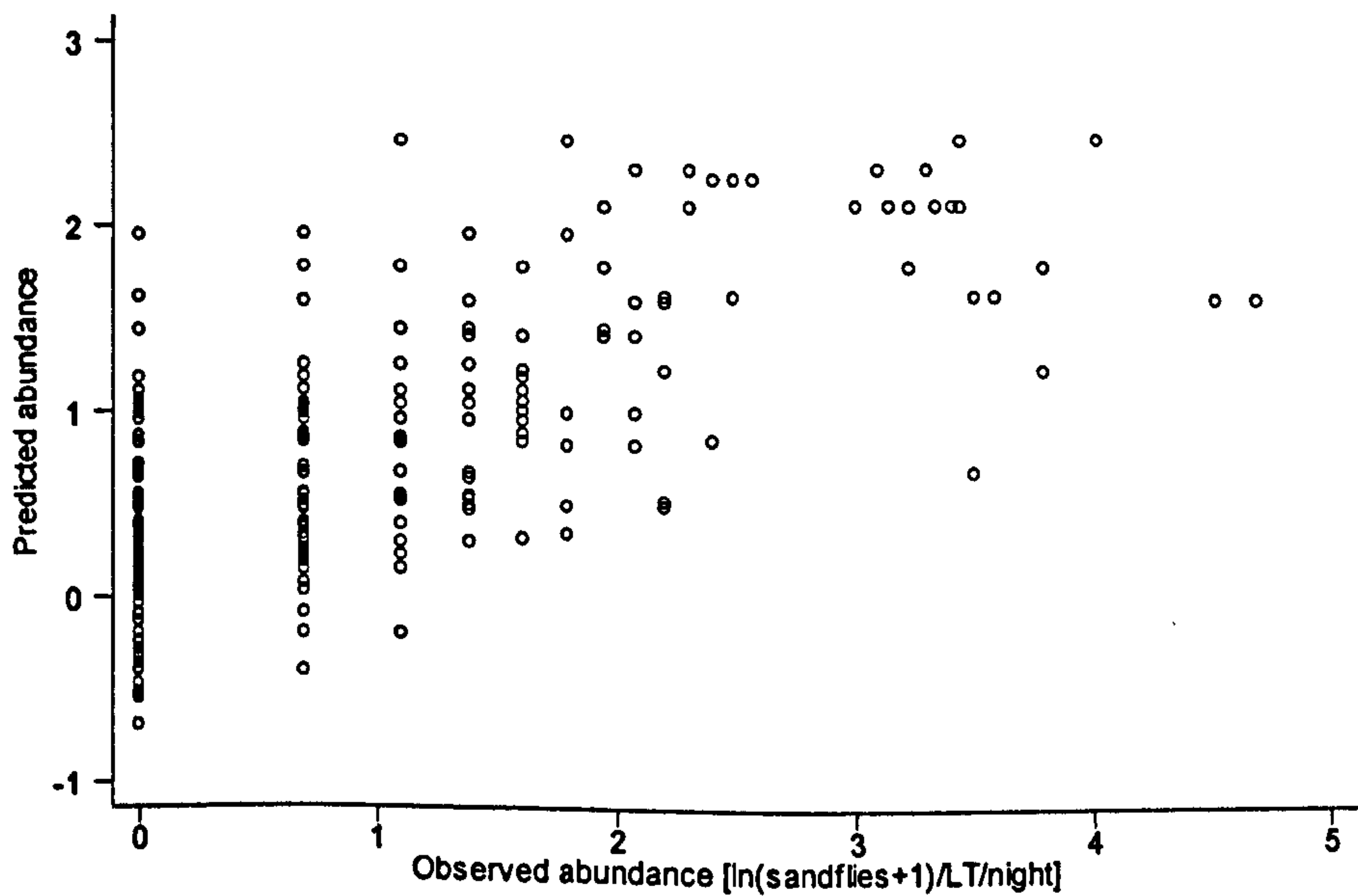
Annexe 19. Normal Quantil-Quantil plot of Anscombe residuals of *Lutzomyia longiflocosa* outdoor abundance.



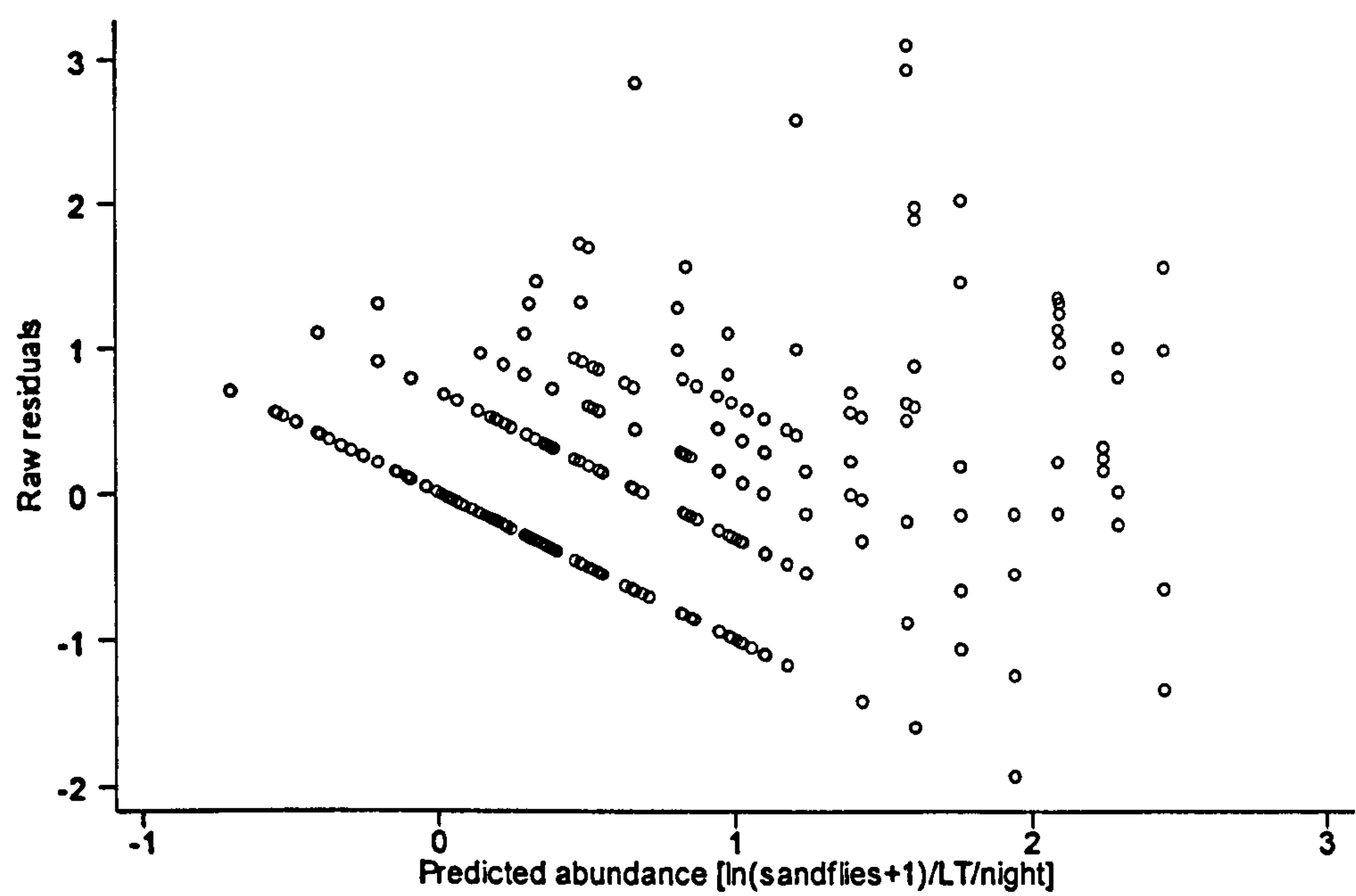
Annexe 20. Significance of ecological determinants for *Lutzomyia nuneztovari* identified by multivariate analysis (MAM with assumption of Normal errors).

Explanatory variable	<i>F</i>	(df ₁ , df ₂)	<i>p</i>
Trap Location			
Position edge or centre	5.24	(1, 390)	0.023
Local determinants			
Slope in the sampling site	7.91	(7, 390)	<0.001
Number of tree strata	7.53	(4, 390)	<0.001
General habitat	12.09	(1, 390)	0.001
Distance to the nearest house	11.64	(1, 390)	0.001
Depth of partially decay litter	3.09	(3, 390)	0.027
Litter cover	2.65	(3, 390)	0.048
Regional determinants			
Soil type	15.33	(10, 390)	<0.001
Slope	3.51	(11, 390)	<0.001
Temperature square	13.78	(1, 390)	<0.001
Temperature	12.67	(1, 390)	<0.001
Rainfall	7.45	(1, 390)	0.007

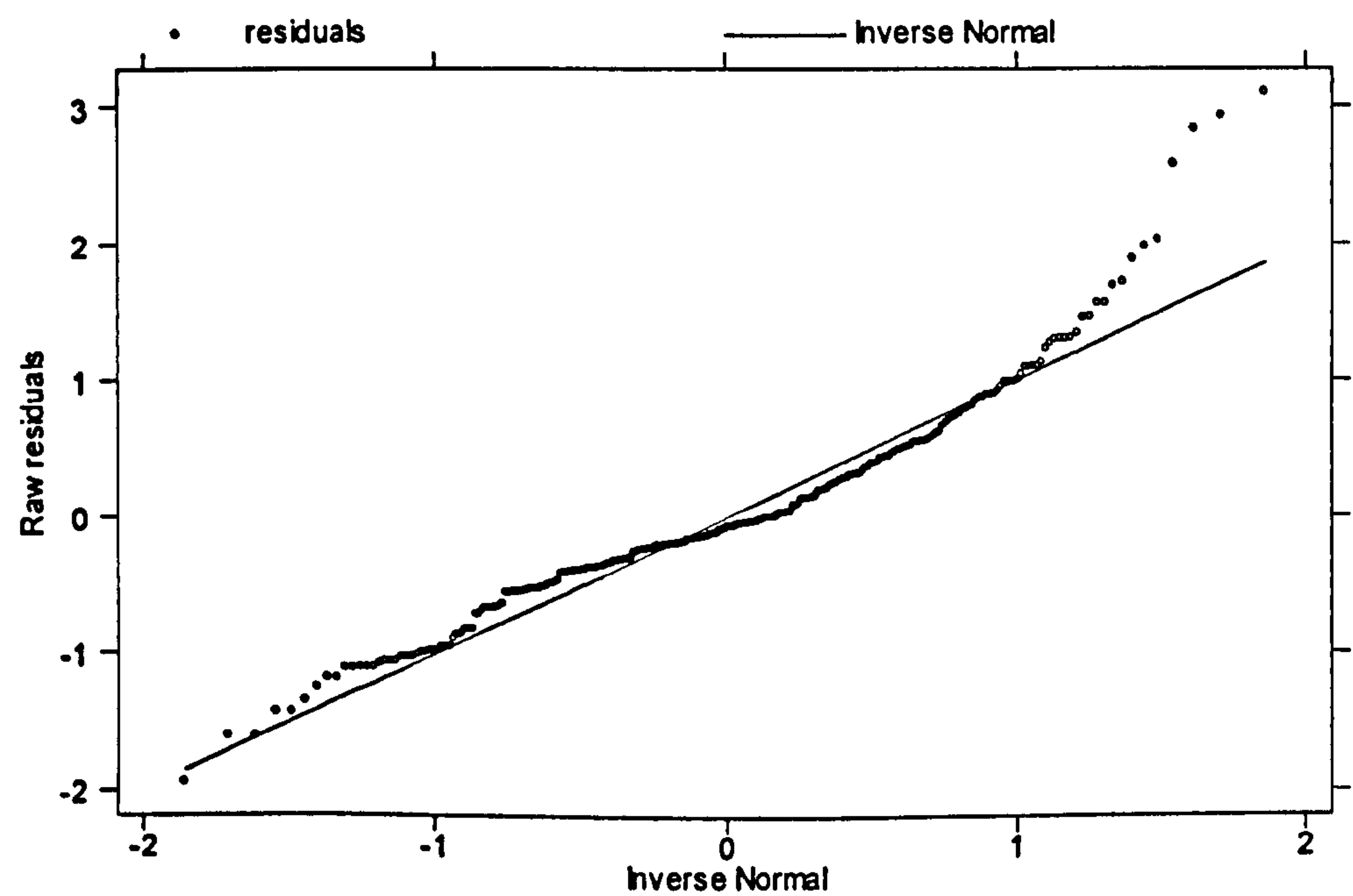
Annexe 21. Observed log transformed outdoors abundance of *Lutzomyia nuneztovari* (collected by CDC light traps) against their predicted value according to the MAM incorporating 12 explanatory variables.



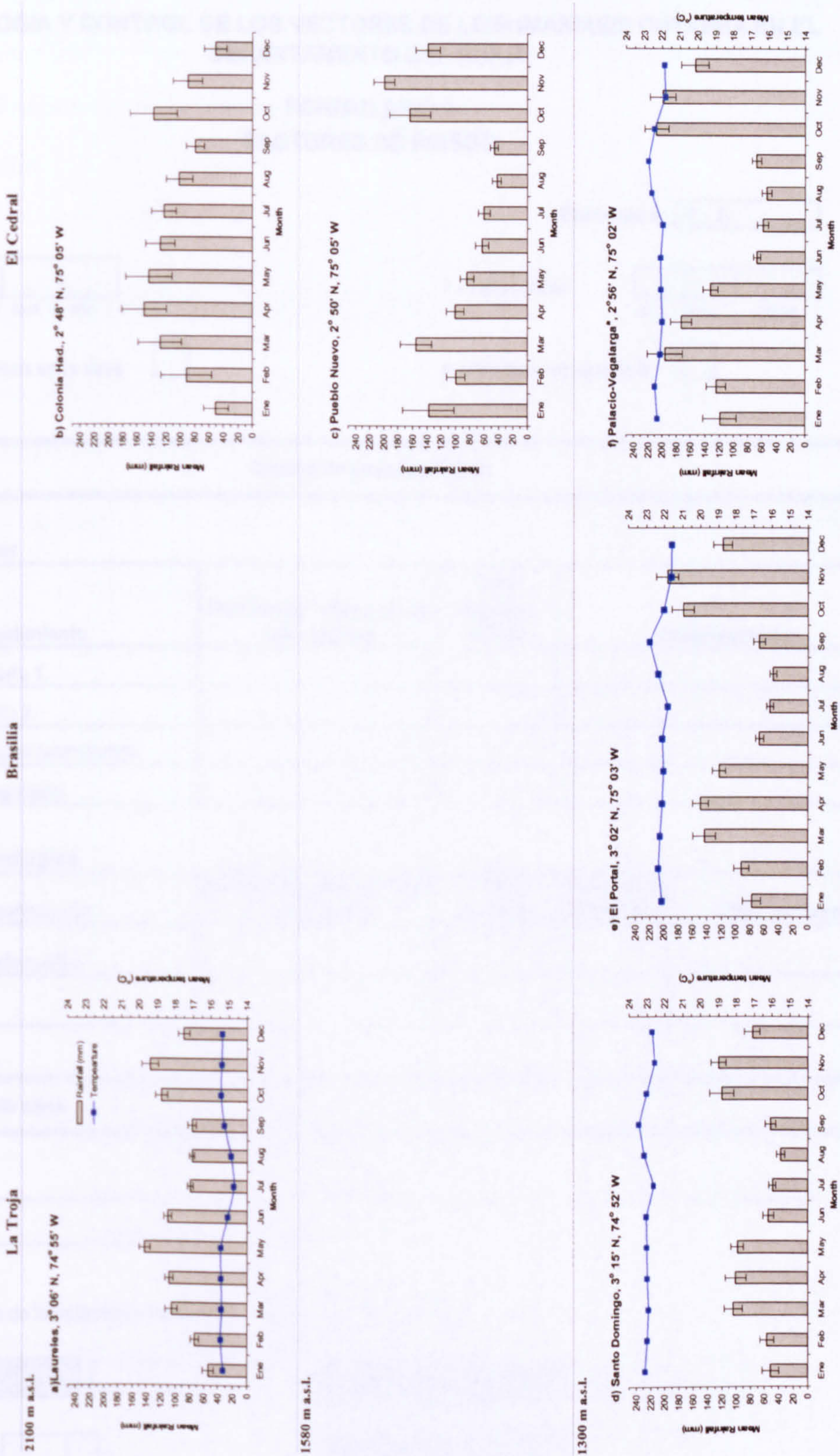
Annexe 22. Log transformed outdoor abundance of *Lutzomyia nuneztovari* predicted by the MAM against the residuals (i.e. observed minus predicted values).



Annexe 23. Normal Quantil-Quantil plot of raw residuals of *Lutzomyia nuneztovari* outdoors abundance.



Annexe 24. Mean total monthly rainfall (and temperature when available) recorded in the nearest climate stations (Data from IDEAM, Colombia) to the three sampled villages, at different altitudes. Number of reported years used to calculate means are as follows: a) Laureles (39 years), b) Hacienda la Colonia (10 years), c) Pueblo Nuevo (17 years), d) Santo Domingo (24 years), e) El Portal (39 years), f) Palacio-Vegalarga (31 years). Error bars corresponds to two standard errors. a Station located at 1100 m a.s.l. .



Annexe 25. Main questionnaire applied for the risk factor trial.

Instituto Nacional de Salud
Laboratorio de Entomología

Secretaría de Salud del Huila
División Laboratorio de Salud Pública

ECOLOGIA Y CONTROL DE LOS VECTORES DE LEISHMANIASIS CUTANEA EN EL DEPARTAMENTO DEL HUILA

FORMULARIO 1

FACTORES DE RIESGO

Encuesta #	F, 1		
------------	------	--	--

1. Fecha

 día / mes / año

2. Código casa

Mun.	Vrda.	Casa #	

3. Número de familias en la casa

☐

4. Familia entrevistada

7

Control de procedimientos

Encuesta familiar

Procedimiento	Número de referencia de cada formato	Total formatos llenos	Observaciones
Entrevista Formulario 1			
Entevista formulario 2			
Remisión para prueba leishmanina			
Remisión para diagnóstico			

Muestreo entomológico

Procedimiento	Número de referencia de la muestra	Total muestras	# aprox. de flebotomos	Observaciones
Trampa CDC intradomiciliar				

I. Ubicación de la casa

5. Municipio _____ 6. Vereda _____

7. Finca o sitio _____

8. Anterior número de identificación de la casa

9. Latitud (grados N)

10. Longitud (grados O)			
-------------------------	--	--	--

11. Error (m)	
---------------	--

12. Altitud (msnm) | | |

Annexe 25. Continued.

II. Datos familiares

13. Hace cuánto tiempo que vive su familia en esta casa ? años meses

En dónde vivían antes ?

14. Departamento 15. Municipio

16. Vereda 17. Finca o sitio

18. Código de la anterior casa (si esta dentro del área de estudio)

Mun.	Verda.	Casa #	

19. Cuántas personas de su familia viven permanentemente en esta casa, incluida usted ?

20. Nombres y edades de estas personas

#	1er y 2do apellidos	Nombres	Sexo (1= fem. 2= mas.)	Fecha de nacimiento
1	(Persona entrevistada)			
Cuántos meses al año pasa esta persona en el hogar ?				
2	(Conyuge () Sí () No)			
Cuántos meses al año pasa esta persona en el hogar ?				
3				
Cuántos meses al año pasa esta persona en el hogar ?				
4				
Cuántos meses al año pasa esta persona en el hogar ?				
5				
Cuántos meses al año pasa esta persona en el hogar ?				
6				
Cuántos meses al año pasa esta persona en el hogar ?				
7				
Cuántos meses al año pasa esta persona en el hogar ?				
8				
Cuántos meses al año pasa esta persona en el hogar ?				

Annexe 25. Continued.

Pg. 3

Encuesta #

F. 1	
------	--

III. Antecedentes de leishmaniasis cutánea

21. Conoce usted la enfermedad llamada leishmaniasis o una enfermedad en la que a las personas les aparece un grano redondeado que se puede convertir en lora, a veces duele y dura por lo menos seis meses para curar ?

1 () Si

2 () No (ir a la pregunta #.....23 y 25)

22. Alguno de los miembros de su hogar tuvo o tiene esta enfermedad ? (de ser posible ver las cicatrices para confirmar si son compatibles con leishmaniasis)

1 () Si

1.1 Cuántos la tienen



1.2 Cuántos la tuvieron

1

1.3 Total



(Diligenciar además el formulario 2)

2 () No

Preguntar a los miembros de la familia presentes si alguno tiene una herida o lesión en la piel o en la nariz. Para los que no estén presentes anotar de acuerdo a lo que responda el jefe de familia o cónyuge

23. Personas con lesiones sospechosas de ser leishmaniasis.....#

11

(Diligenciar además el formulario 2 si se presentan casos sospechosos)

IV. Control de la leishmaniasis

24. Qué medidas para evitar la leishmaniasis se practican en su hogar ?

1 () Toldillos

1.1 Tamaño de ojo de maya:

1.2 Cantidad

1 () Grande (para mosquitos).....

4

2 () Pequeño (para mantablanca).....

7

1.3 En qué fecha aproximada empezaron a usarlos ?

--	--

mes / año

1.4 Con qué frecuencia lo usan ?

1 () Todo el tiempo

2 () En época de abundancia del mantablanca o capotillo. Cuál ?

3 () Otra. Cuál ?

2 () Fumigación con veneno

2.1 Nombre del veneno:

2.2 En qué fecha aproximada empezaron a usarlos ?

--	--	--

mes / año

2.3 Con qué frecuencia lo usan ?

1 () Todo el tiempo

2 () En época de abundancia del mantablanca o capotillo. Cuál ?

3 () Otra. Cuál ?

Annexe 25. Continued.

Pg. 4

Encuesta #

F	1			
---	---	--	--	--

3 () Otra 1

3.1 Nombre: _____

3.2 En qué fecha aproximada empezaron a usarla ?

--	--	--	--

mes / año

3.3 Con qué frecuencia la usan ?

1 () Todo el tiempo

2 () En epoca de abundancia del mantablanca o capotillo. Cuál ? _____

3 () Otra. Cuál ? _____

4 () Otra 2

4.1 Nombre: _____

4.2 En qué fecha aproximada empezaron a usarla ?

--	--	--	--

mes / año

4.3 Con qué frecuencia la usan ?

1 () Todo el tiempo

2 () En epoca de abundancia del mantablanca o capotillo. Cuál ? _____

3 () Otra. Cuál ? _____

5 () Ninguna

25. Ha sido fumigada su casa por funcionarios de la Secretaria de Salud del Huila ?

1 () Sí, en qué fecha fue la última fumigación (mes y año)?

--	--	--	--

mes / año

2 () No

V. Conocimiento del vector

26. Conoce usted al mantablanca ?

1 () Sí

2 () No (ir a la pregunta #.....29)

27. Qué enfermedad causa el mantablanca ?

1 () Paludismo

2 () Leishmaniasis

3 () Dengue

4 () Otra. Cuál ? _____

5 () Ninguna

Annexe 25. Continued.

28. Qué medidas practican en su casa para evitar que el mantablanca los pique ?

1 () Toldillos

1.1 Tamaño de ojo de maya:

1 () Grande (para mosquitos).....

2 () Pequeño (para mantablanca).....

1.2 Cantidad

--

--

1.3 En qué fecha aproximada empezaron a usarlos ?

--	--

mes / año

1.4 Con qué frecuencia lo usan ?

1 () Todo el tiempo

2 () En epoca de abundancia del mantablanca o capotillo. Cuál ? _____

3 () Otra. Cuál ? _____

2 () Fumigación con veneno

2.1 Nombre del veneno: _____

2.2 En qué fecha aproximada empezaron a usarlos ?

--	--

mes / año

2.3 Con qué frecuencia lo usan ?

1 () Todo el tiempo

2 () En epoca de abundancia del mantablanca o capotillo. Cuál ? _____

3 () Otra. Cuál ? _____

3 () Otra 1

3.1 Nombre: _____

3.2 En qué fecha aproximada empezaron a usarla ?

--	--

mes / año

3.3 Con qué frecuencia la usan ?

1 () Todo el tiempo

2 () En epoca de abundancia del mantablanca o capotillo. Cuál ? _____

3 () Otra. Cuál ? _____

4 () Otra 2

4.1 Nombre: _____

4.2 En qué fecha aproximada empezaron a usarla ?

--	--

mes / año

4.3 Con qué frecuencia la usan ?

1 () Todo el tiempo

2 () En epoca de abundancia del mantablanca o capotillo. Cuál ? _____

3 () Otra. Cuál ? _____

5 () Ninguna

6 () Las mismas que se mencionaron para evitar la leishmaniasis

VI. Características del domicilio

29. Tipo de vivienda:

1 () Casa

2 () Rancho

Annexe 25. Continued.

30. Materiales de las paredes:

- 1 () Ladrillo

2 () Bahareque
- 3 () Madera

4 () Otro. Cuál ? _____

31. Terminado de la pared (pañetado):

- 1 () Sí
- 2 () No

32. Presencia de grietas en la pared:

- 1 () Ninguna
- 2 () Pocas (del 1% al 30 % del área)
- 3 () Regular (del 31% al 60%)
- 4 () Muchas (del 61% al 100%)

33. Material del techo:

- 1 () Teja de zinc
- 2 () Palma o paja
- 3 () Otro. Cuál ? _____

34. Presencia de cielo raso:

- 1 () Sí
- 2 () No, (ir a la pregunta #.....36)

35. Materiales y acabado del cielo raso:

- 1 () Tabla, sin espacios entre tablas
- 2 () Tabla, con hueco de acceso para secado de café
- 3 () Otro. Cuál ? _____

36. Aperturas permanentes de la casa (área, m²)

_____	_____	_____	Total..... <table border="1"><tr><td></td><td></td></tr></table>		
_____	_____	_____			

37. Alumbrado eléctrico:

- 1 () Sí. Hace cuantos años ?

--	--
- 2 () No

38. Cuántas piezas se utilizan para dormir en su hogar ?

--	--

39. En dónde duermen ?

- 1 () Cama sencilla (hasta 1.20m).....#

--
- 2 () Cama doble (mayor a 1.20m).....#

--
- 3 () Cuna o corral.....#

--
- 4 () Otro. Cuál ? _____..#

--

40. Distancia a la casa más cercana (m)

41. Número de casas dentro de 100m a la redonda

VII. Características del peridomicilio (Hasta 50m de radio alrededor de la vivienda)

42. Qué animales domésticos hay en su vivienda ? (anotar número de cada especie)

			#	Duermen en covertizos ?	A qué distancia de la vivienda duermen (m) ?
1. Perros	1 () Sí	2 () No	<div></div>	1 () Sí 2 () No	<div></div>
2. Gatos	1 () Sí	2 () No	<div></div>	1 () Sí 2 () No	<div></div>
3. Gallinas	1 () Sí	2 () No	<div></div>	1 () Sí 2 () No	<div></div>
4. Marranos	1 () Sí	2 () No	<div></div>	1 () Sí 2 () No	<div></div>
5. Vacas	1 () Sí	2 () No	<div></div>	1 () Sí 2 () No	<div></div>
6. Equinos	1 () Sí	2 () No	<div></div>	1 () Sí 2 () No	<div></div>
7. Otro	1 () Sí	2 () No	<div></div>	1 () Sí 2 () No	<div></div>

Cuál ? (para animales silvestres anotar si provienen de la misma área o de otra)

43. Número de árboles según altura:

1 () 2 - 5m	<div></div>	3 () 10.1 - 20m	<div></div>
2 () 5.1 - 10m	<div></div>	4 () >20m	<div></div>

44. Cobertura total de árboles (%)

45. Presencia de plantas de platano o banano

1 () No

2 () Regular (1 - 10 plantas)

3 () Mucho (más de 10 plantas)

VIII. Características del Extradomicilio (hasta 300 m alrededor de la vivienda)

46. Qué animales de monte o silvestres se pueden encontrar en cercanías a su vivienda (dentro de 300m a la redonda) ?

1 () Chucha	4 () Armadillo
2 () Zorro	5 () Otros, cuáles:
3 () Ratón	

47. H bitats circundantes

	(%)	Edad (a�os)	Tiempo que ha estado este tipo de vegetaci�n en el sitio (a�os)
1 () Bosque	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>
2 () Cafetal tradicional con sombr�o	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>
3 () Otro cultivo con sombr�o arb�reo	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>
Cu�l ? _____			
4 () Cafetal semisombra	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>
5 () Cafetal al sol	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>
6 () Pastizal	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>
7 () Otros cultivos:	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>	<div></div> <div></div> <div></div>
Cu�les ? _____			
Total.....	<div></div> <div></div> <div></div>		

Observaciones

Formulario diligenciado por: _____

- Nota:**
- 1) Los **numerales subrayados** deben **diligenciarse por el encuestador** con base en observaciones directas.

2) **No diligenciar** los numerales **sombreados**.

Annexe 26. Questionnaire applied for each suspected CL case in the risk factor trial.

Instituto Nacional de Salud
Laboratorio de Entomología

Secretaría de Salud del Huila
División Laboratorio de Salud Pública

ECOLOGIA Y CONTROL DE LOS VECTORES DE LEISHMANIASIS CUTANEA EN EL
DEPARTAMENTO DEL HUILA

FORMULARIO 2
CASOS CONFIRMADOS O POSIBLES DE LEISHMANIASIS CUTANEA

1. Fecha

día / mes / año

Encuesta #

F

2

2. Código de referencia

Mun. Vrda. Casa #

3. F1 de referencia

F

1

I. Información general del paciente

4. # fam.

5. Apellidos

Nombres

6. Sexo
(1= fem.
2= mas.)

7. Fecha de nacimiento

8. Información suministrada por

1 () Paciente

2 () Persona entrevistada para el formulario 1

3 () Otra. Quién ?

9. Qué edad tenía cuando contrajo la enfermedad ?

años

meses

10. En dónde vivía durante esa época ?

1 () La misma casa (ir a la pregunta #.....17)

2 () Otra

11. Departamento

12. Municipio

13. Vereda

14. Finca o sitio

15. Propietario

16. Código de la anterior casa (si pertenece al área de estudio)

Mun. Vrda. Casa #

17. El paciente presenta:

1 () Lesión activa (ir a la pregunta #..... 18)

2 () Cicatriz (ir a la pregunta #.....25)

II. Posible caso activo

18. Ha consultado a alguna persona ?

1 () Sí

2 () No (ir a la pregunta #.....23)

19. A quién consulto ?

1 () Médico

3 () Promotor

2 () Enfermera

4 () Otra, quién ?

Annexe 26. Continued.

Pg. 2

Encuesta #

F 2	
-----	--

20. En dónde consultó ? _____

21. Se le tomó una muestra de su lesión o grano ?

1 () SI

2 () No

22. Cuál fue el diagnóstico ?

1 () Leishmaniasis

2 () Otra, cuál ? _____

23. Qué tratamiento se está aplicando?

1 () Inyecciones de Glucantime

2 () Otro, cuál? _____

3 () Ninguno

24. Confirmación del caso activo como leishmaniasis cutánea

1 () Si

2 () No, en duda (Ir a pregunta #....30)

3 () Descartado

III. Caso curado

25. Consultó a alguna persona ?

1 () Si

2 () No (Ir a la pregunta #....28)

26. A quién consulto ?

1 () Médico

3 () Promotor

2 () Enfermera

4 () Otra, quién ? _____

27. Cuál fue el diagnóstico ?

1 () Leishmaniasis

2 () Otra, Cuál ? _____

28. Qué tratamiento recibió ?

1 () Inyecciones de Glucantime

2 () Otro, cuál? _____

3 () Ninguno

29. Confirmación del caso curado como leishmaniasis cutánea

1 () SI

2 () No, en duda (Ir a pregunta #....34)

IV. Remisión de pacientes

Possible caso activo

30. Remisión para diagnóstico número:

DIAG

--	--

Annexe 26. Continued.

Pg. 3

Encuesta #

F	2			
---	---	--	--	--

31. Tipo de diagnóstico:

- 1 () Parasitológico

2 () Histopatológico

3 () Inmunológico
- 4 () Clínico

5 () Montenegro. Diámetro de la induración (mm)

--	--

32. Resultado:

- 1 () Positivo
- 2 () Negativo

33. Confirmación de la lesión como caso de leishmaniasis:

- 1 () Sí
- 2 () No

Posible caso curado

34. Remisión para prueba de montenegro Número: MONT

--	--

35. Diámetro de la induración (mm):

--

 x

--

36. Resultado:

- 1 () Positivo
- 2 () Negativo

37. Confirmación de la cicatriz como posible caso de leishmaniasis:

- 1 () Sí
- 2 () No

Observaciones

--

Formulario diligenciado por: _____

Nota:

- 1) Los **numerales subrayados** deben **diligenciarse por el encuestador** con base en observaciones directas.
- 2) **No diligenciar** los numerales **sombreados**.

Annexe 27. Form to monitor the evolution of suspected new CL cases.

Instituto Nacional de Salud
Laboratorio de Entomología

Secretaría de Salud del Huila
División Laboratorio de Salud Pública

ECOLOGIA Y CONTROL DE LOS VECTORES DE LEISHMANIASIS CUTANEA EN EL
DEPARTAMENTO DEL HUILA

REMISION PARA DIAGNOSTICO DE LEISHMANIASIS CUTANEA

(4 Copias: paciente, centro de salud, SSDH y archivo)

Fecha

día / mes / año

Código de remisión

DIAG

Apellidos

Nombres

Sexo
(1= fem.
2= mas.)

años

Favor presentarse en:

1 () Centro de Salud Cándido en Neiva (Dr. Ramiro Sánchez)

2 () Centro de Salud de Tello (Dr. César González)

3 () Hospital de Baraya (Dr. Alvaro Ruiz)

para la realización de las pruebas diagnósticas necesarias con el fin de determinar si presenta leishmaniasis cutánea e iniciar el tratamiento adecuado a la mayor brevedad, de confirmarse la enfermedad.

32

Annexe 28. Description of variables examined as potential risk factors for indoors sandfly abundance and cutaneous leishmaniasis cases.

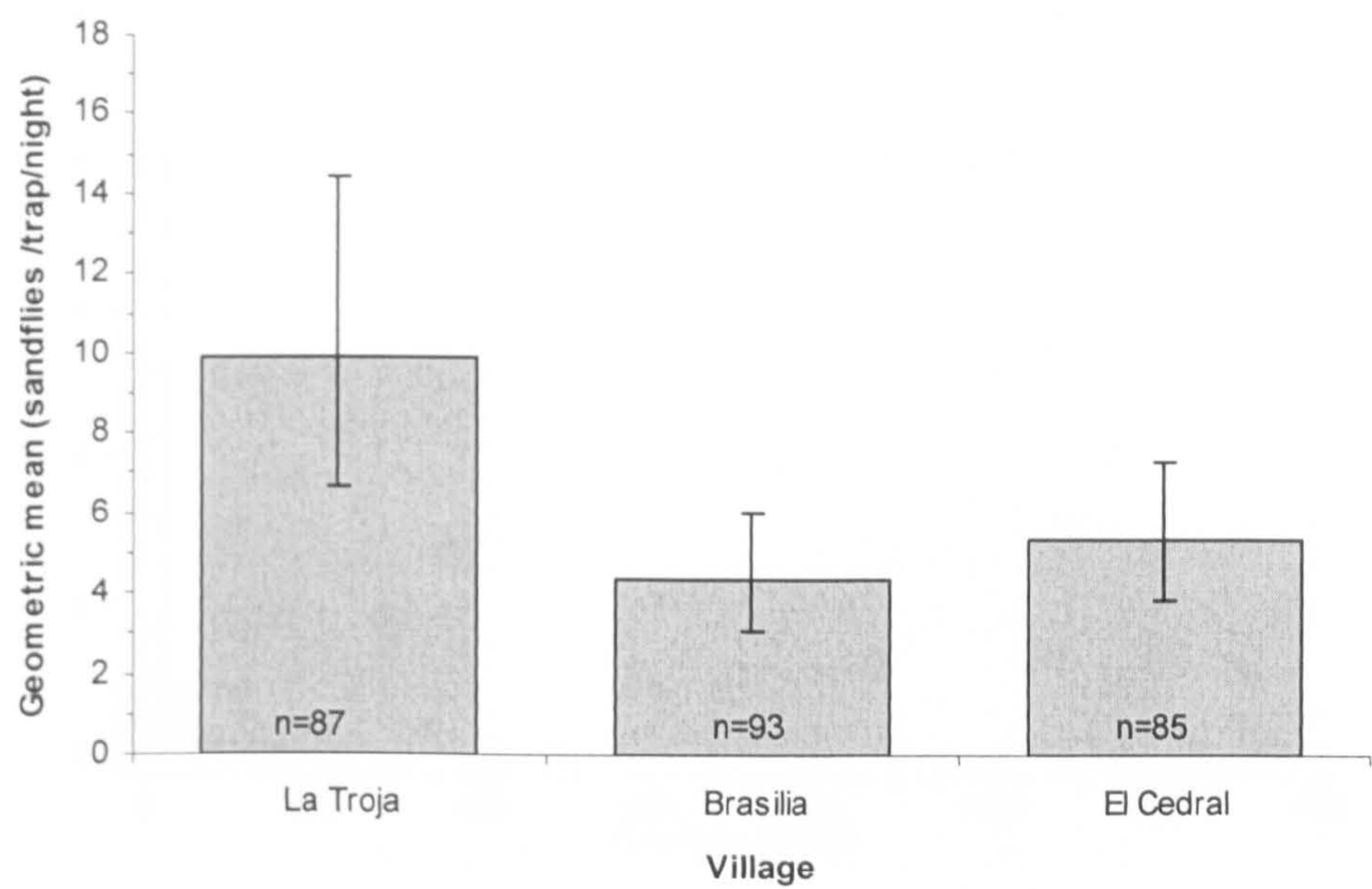
Variable	n	Mean or %	SD	Min	Max
Village (n = 271 houses)					
Brasilia	98	36.2			
La Troja	87	32.1			
El Cedral	86	31.7			
Altitude (m a.s.l)	271	1655	147	1310	2180
Features of the habitat surrounding each house					
Mean percentage of vegetation cover (within 300 m radius)					
Grass and other herbaceous plants	271	42.7	28.5	0	100
Unshaded coffee	271	29.5	31.8	0	100
Forest	271	12.8	11.1	0	60
Semi shaded coffee	271	7.8	18.1	0	85
Traditional coffee	271	3.1	11.5	0	95
Banana ^a	271	2.2	6.2	0	40
Sugar cane	271	1.4	5.8	0	65
Vegetation (within 50 m radius)					
Number of trees 2 - 10 m height	270	24.3	13.0	0	103
Number of trees > 10 m height	269	6.7	8.6	0	60
Percentage of houses with banana plants ^a (n = 271)					
0	35	12.9			
1 - 10	12	4.4			
> 10	224	82.7			
Percentage of cover (trees > 2 m height)	269	13.9	11.6	0	60
Distance to the nearest house (m)	270	147	107	0	500
Number of Houses within 100 m	270	1.1	1.8	0	9
Percentage of houses with animal shelters (within 200 m radius, n = 271)					
No	109	40.2			
Yes	162	59.8			
House features					
Percentage of housing type (n = 271)					
House	226	83.4			
Hut	45	16.6			
Percentage with wall type (n = 267)					
"Bahareque"	156	58.4			
Brick	51	19.1			
Wood	45	16.9			
Stone and cement	15	5.6			

Annexe 28. Continued.

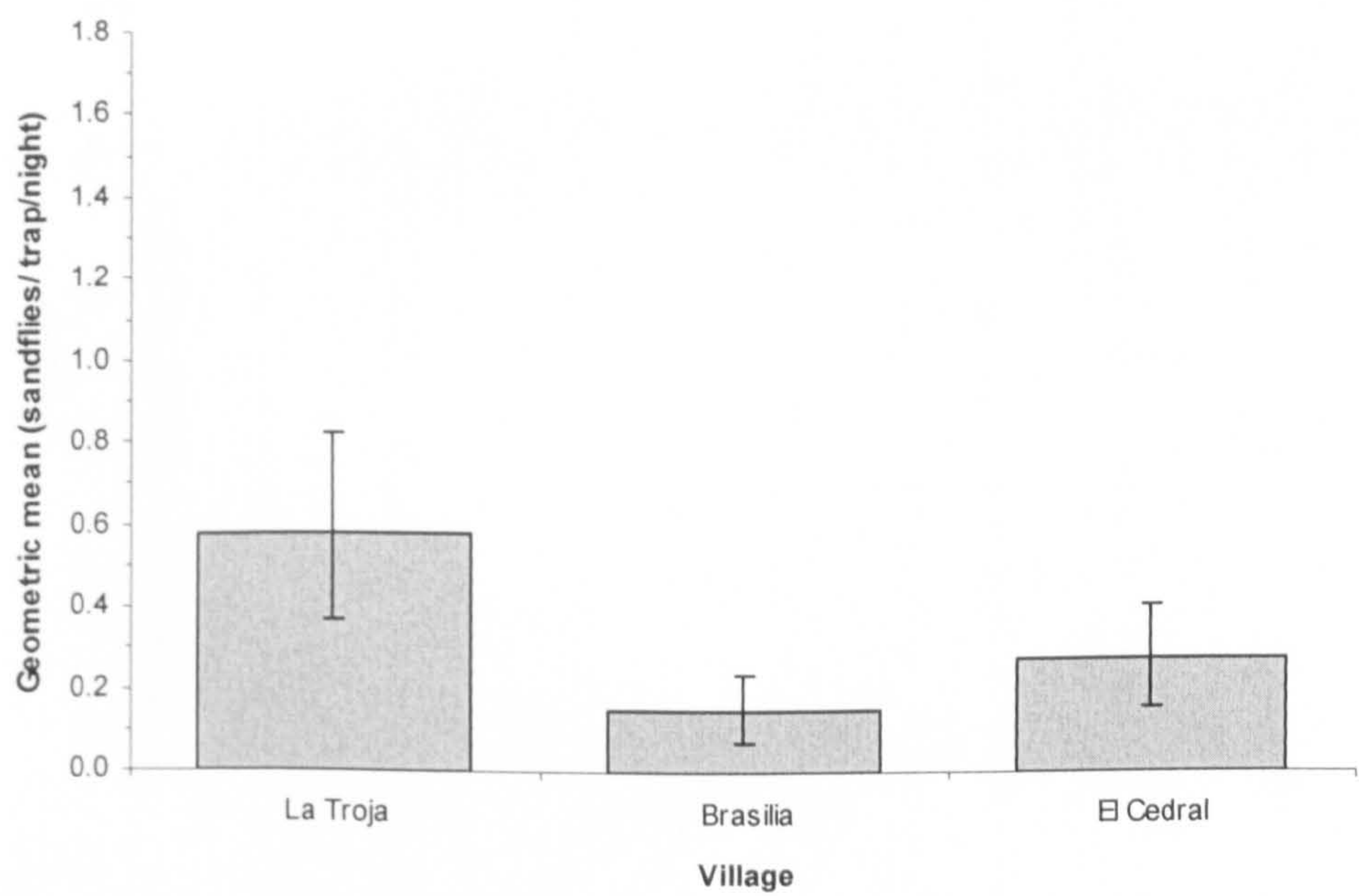
Variable	n	Mean or %	SD	Min	Max
Percentage with wall cracks (n = 265)					
0 %	149	56.2			
1 - 30%	65	24.5			
31 - 60%	23	8.7			
>60	28	10.6			
Percentage with smooth walls (n = 260)					
No	106	40.8			
Yes	154	59.2			
Percentage with ceiling type (n = 260)					
No ceiling	93	35.8			
Close plank	70	26.9			
Plank with spaces	63	24.2			
Close plank and hole	34	13.1			
Total openings (m ²)	255	5.8	6.8	0	31.9
Time with electricity service (years)	258	9.1	7.3	0	30
Number of potential hosts					
Total persons per house	271	5.4	2.7	1	18
Total domestic animals per house (within 200 m radius)					
Chickens	270	12.2	10.0	0	90
Dogs	271	1.5	1.3	0	5
Cows	270	0.94	3.6	0	25
Pigs	269	0.48	1.3	0	13
Equines (horse, donkey, mule)	270	0.43	1.0	0	6
Cats	271	0.25	0.6	0	4
Vector control measures at household level					
Percentage of houses using smoke (n = 268)					
No	128	47.8			
Yes	140	52.2			
Percentage of houses using spraying with insecticides (n = 268)					
No	198	73.9			
Yes	70	26.1			
Percentage of houses using spraying with non-insecticidal substances (n = 268)					
No	218	81.3			
Yes	50	18.7			
Percentage of houses using bednets	269	30.1			
Number of bednets per house where bednets were used	81	1.8	1.0	0	5

* Includes some plantain plants.

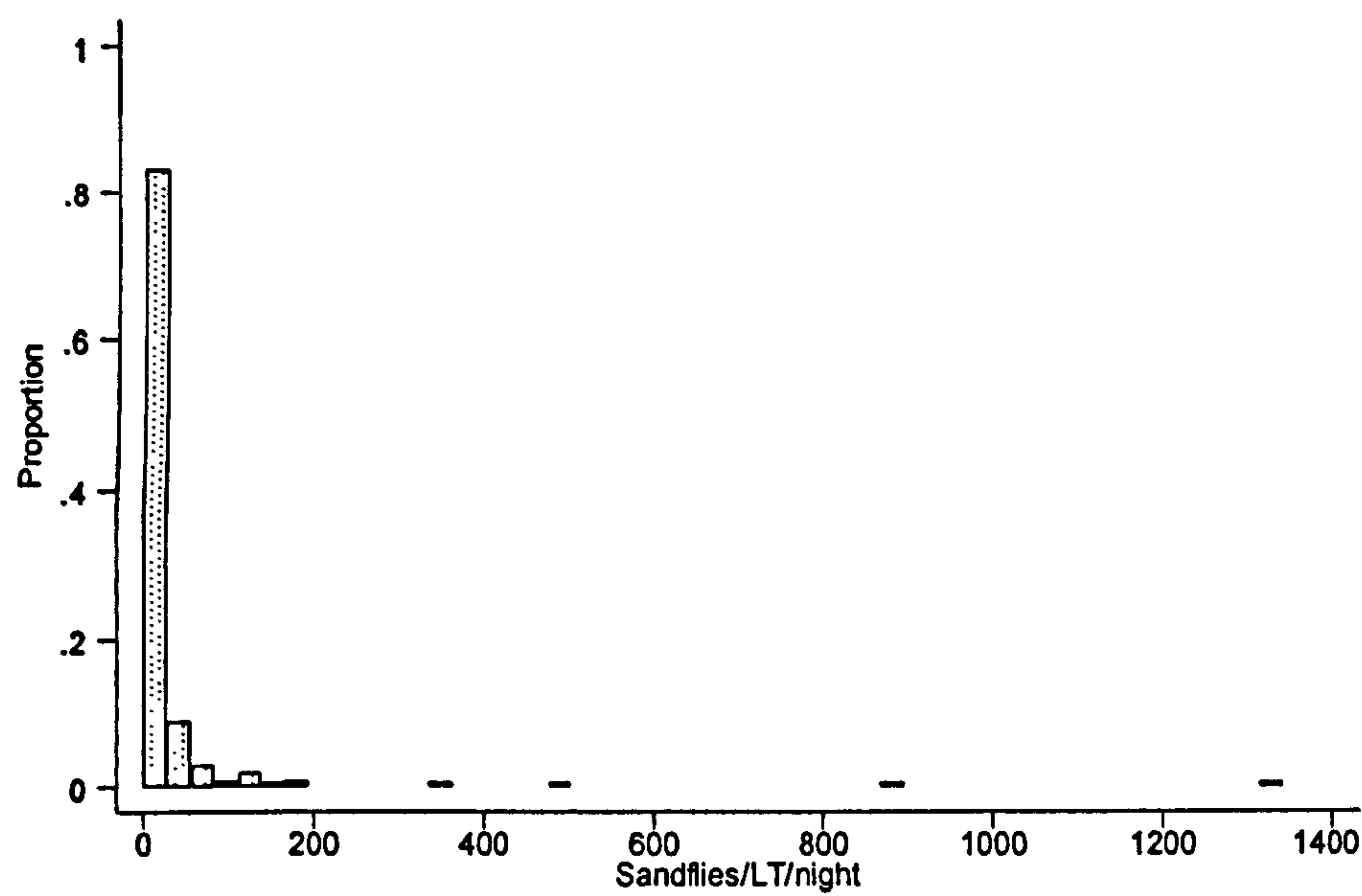
Annexe 29. The relationship between village and *Lutzomyia longiflocosa* abundance inside houses (as measured by CDC light traps).



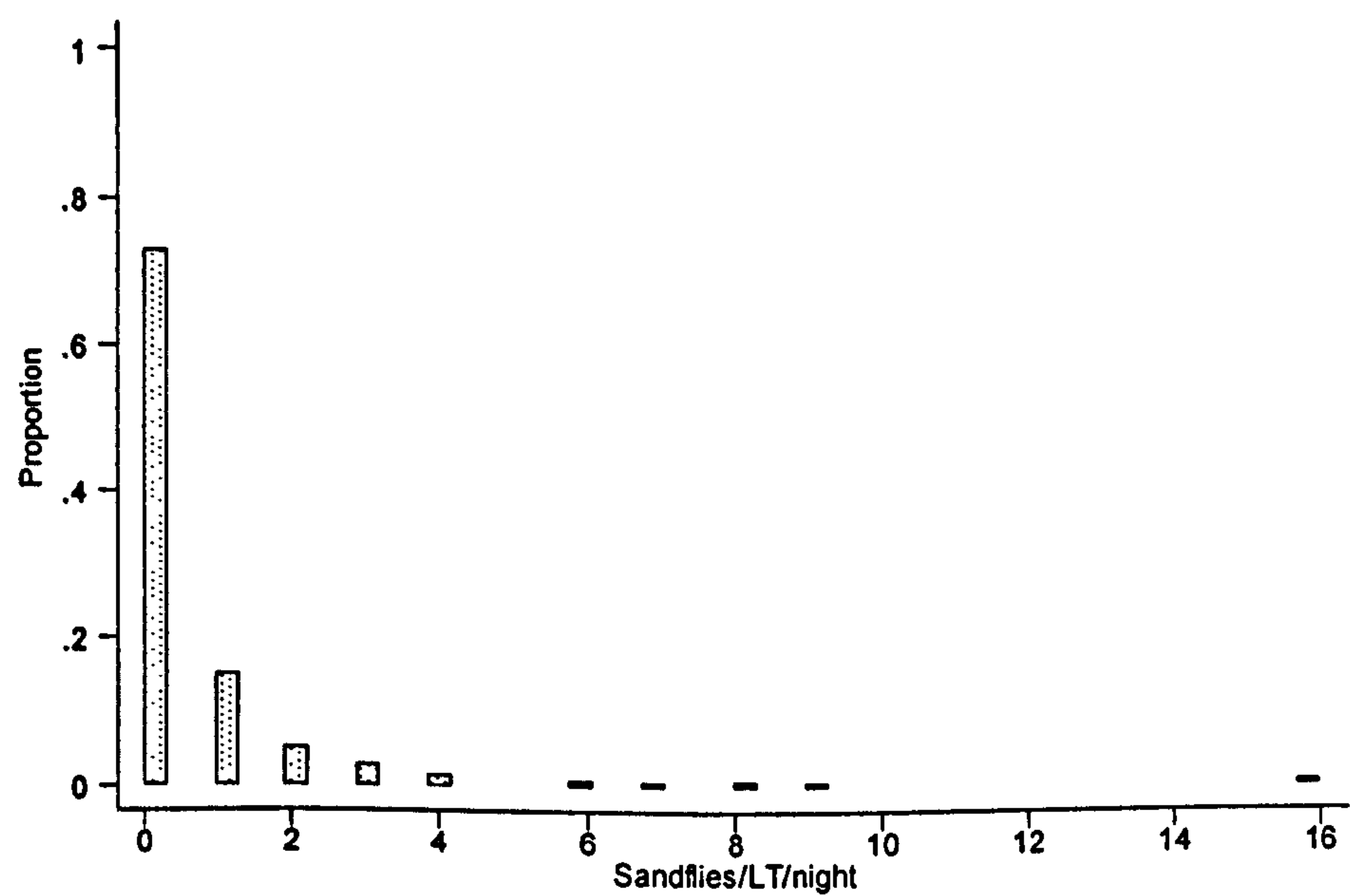
Annexe 30. The relationship between village and *Lutzomyia nuneztovari* abundance inside houses (as measured by CDC light traps).



Annexe 31. Frequency distribution of the raw data of *Lutzomyia longiflocosa* (n = 265, total sandflies = 7162).



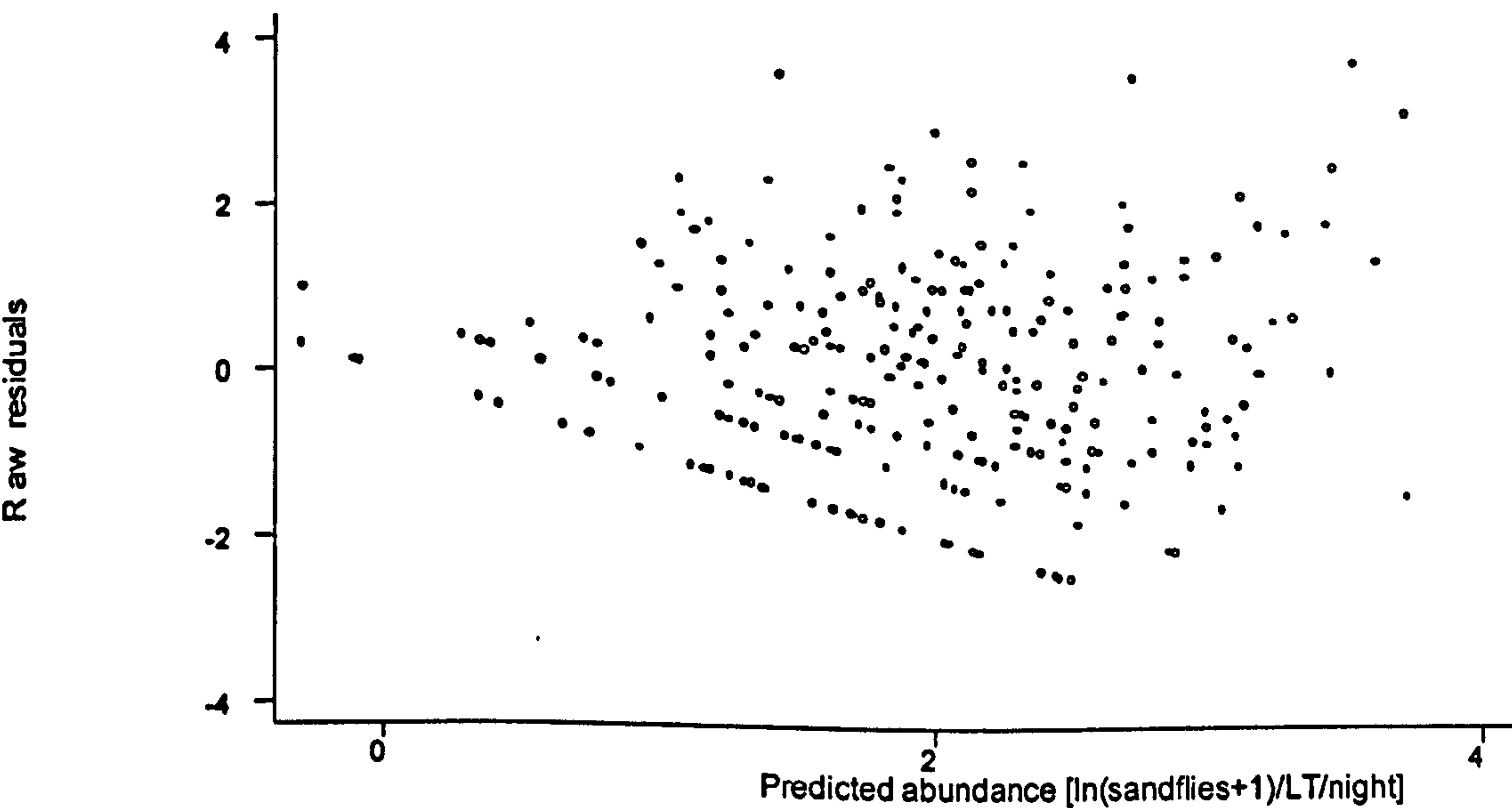
Annexe 32. Frequency distribution of the raw data of *Lutzomyia nuneztovari* (n = 265, total sandflies = 163).



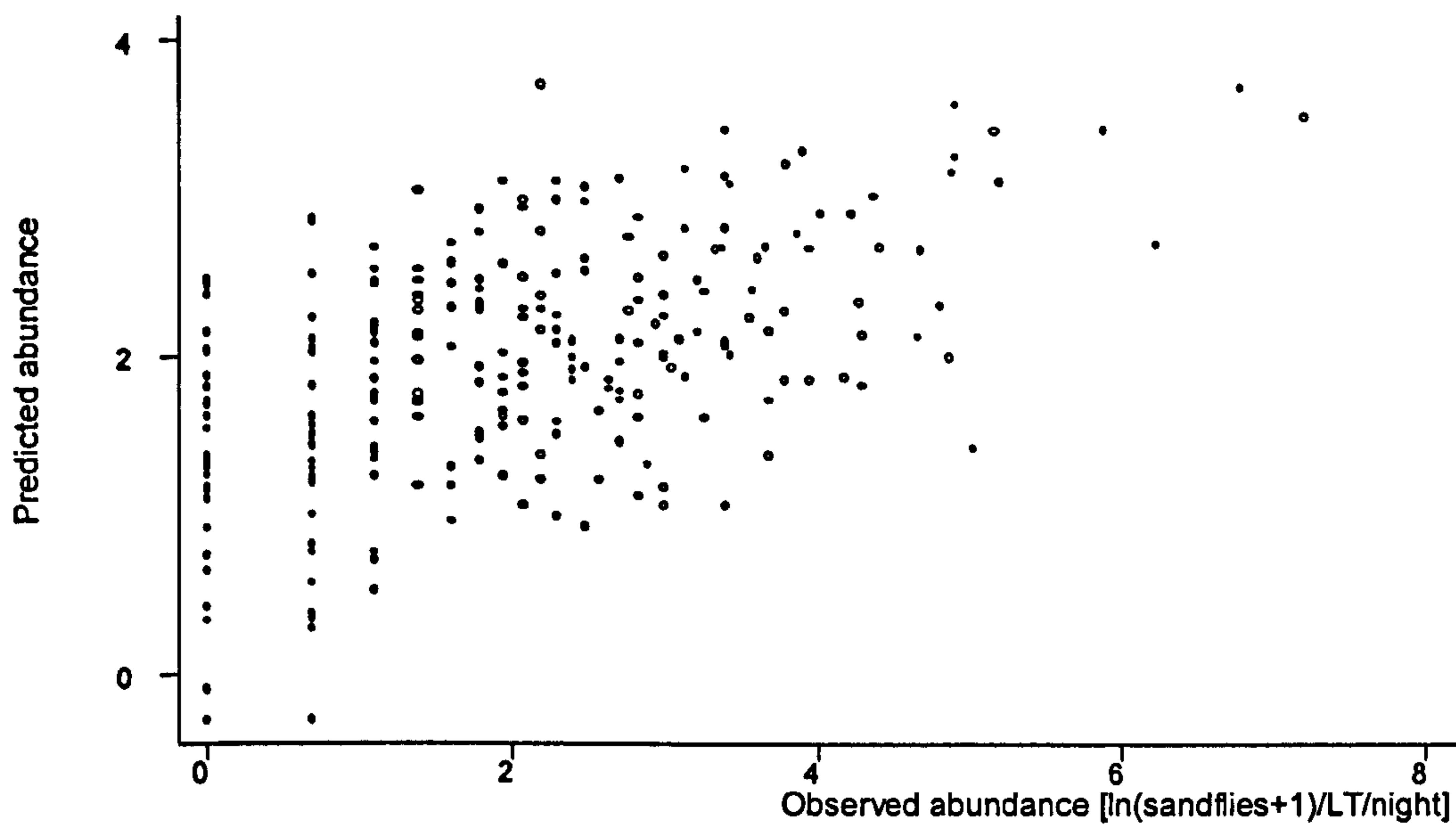
Annexe 33. Significance and explanatory power (r^2 , in percentage) of risk factors for *Lutzomyia longiflocosa* identified by multivariate analysis (MAM with assumption of Normal errors).

Varname	<i>F</i>	(df ₁ , df ₂)	<i>p</i>	<i>r</i> ²
Village	15.16	(2, 250)	<0.001	8.9
Altitude	3.10	(7, 250)	0.004	6.4
Potential hosts				
Number of dogs (within 200 m)	15.58	(1; 250)	<0.001	4.6
Number of persons per house	12.74	(1; 250)	<0.001	3.8
Surrounding habitats features				
Number of houses (within 100 m)	5.29	(2, 250)	0.006	3.1
Percentage of grass (within 300 m)	9.41	(1; 250)	0.002	2.8
Total				26.3

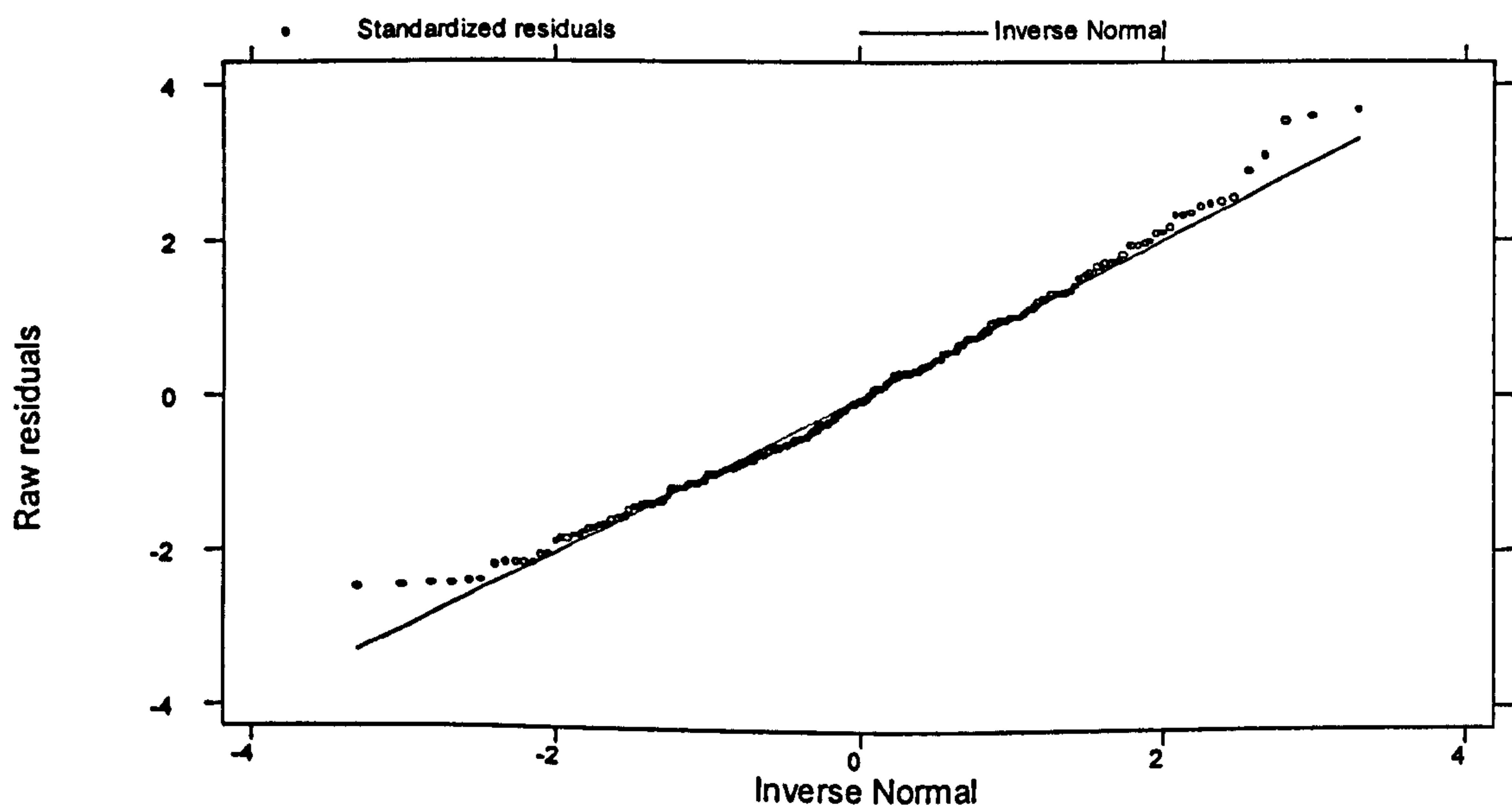
Annexe 34. Log transformed indoor abundance of *Lutzomyia longiflocosa* predicted by the MAM against the residuals (i.e. observed minus predicted values).



Annexe 35. Observed log transformed indoors abundance of *Lutzomyia longiflocosa* (collected with CDC light traps) against their predicted value according to the MAM incorporating altitude, village, number of persons in the house, number of dogs, percentage of grass (within 300 m radius), and number of houses within 100 m radius as explanatory variables.



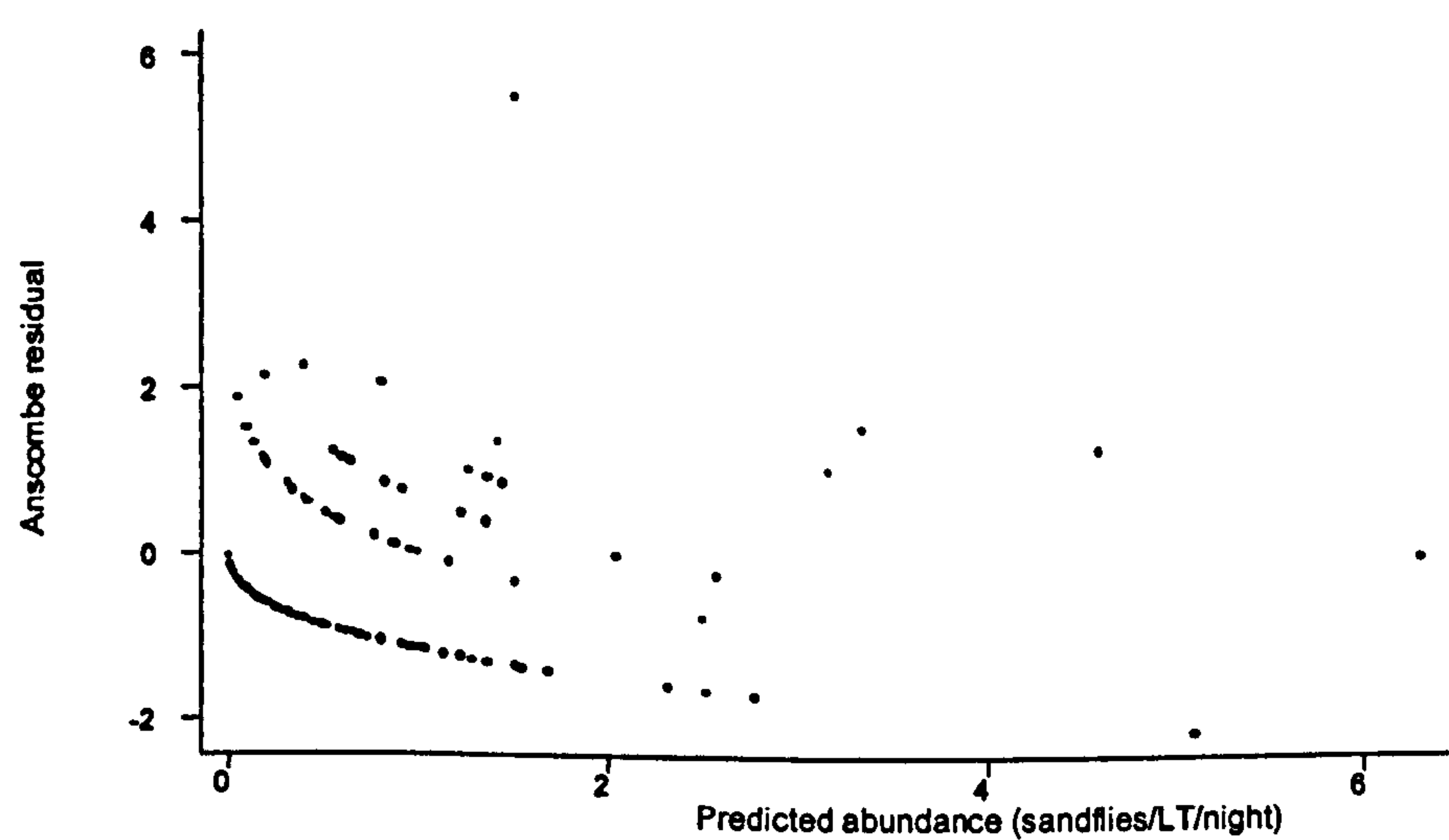
Annexe 36. Normal Quantil-Quantil plot of raw residuals of *Lutzomyia longiflocosa* indoor abundance.



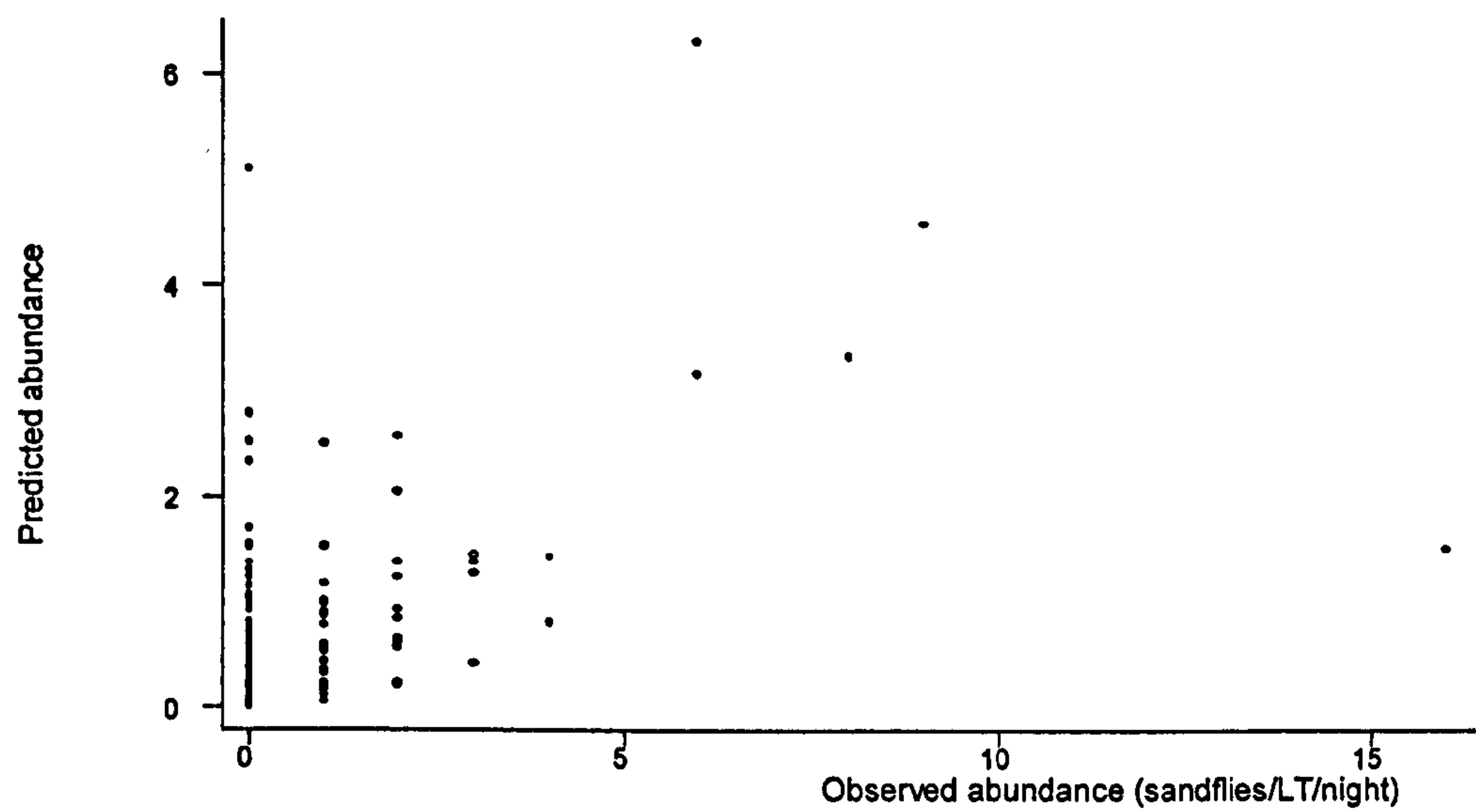
Annexe 37. Significance and explanatory power (r^2 , in percentage) of risk factors for *Lutzomyia nuneztovari* identified by multivariate analysis (MAM with assumption of negative binomial errors).

Variance	χ^2	(df)	p	r^2
Village	27.1	(2)	<0.001	5.2
House features				
Type of ceiling	41.44	(7)	<0.001	7.9
Potential hosts				
Number of dogs (within 200 m)	18.34	(1)	<0.001	3.5
Number of pigs (within 200 m)	12.01	(2)	0.002	2.3
Number of persons per house	4.63	(1)	0.031	0.9
Surrounding habitats features				
Number of banana plants (within 50 m)	6.98	(1)	0.008	1.3
Total				16.9

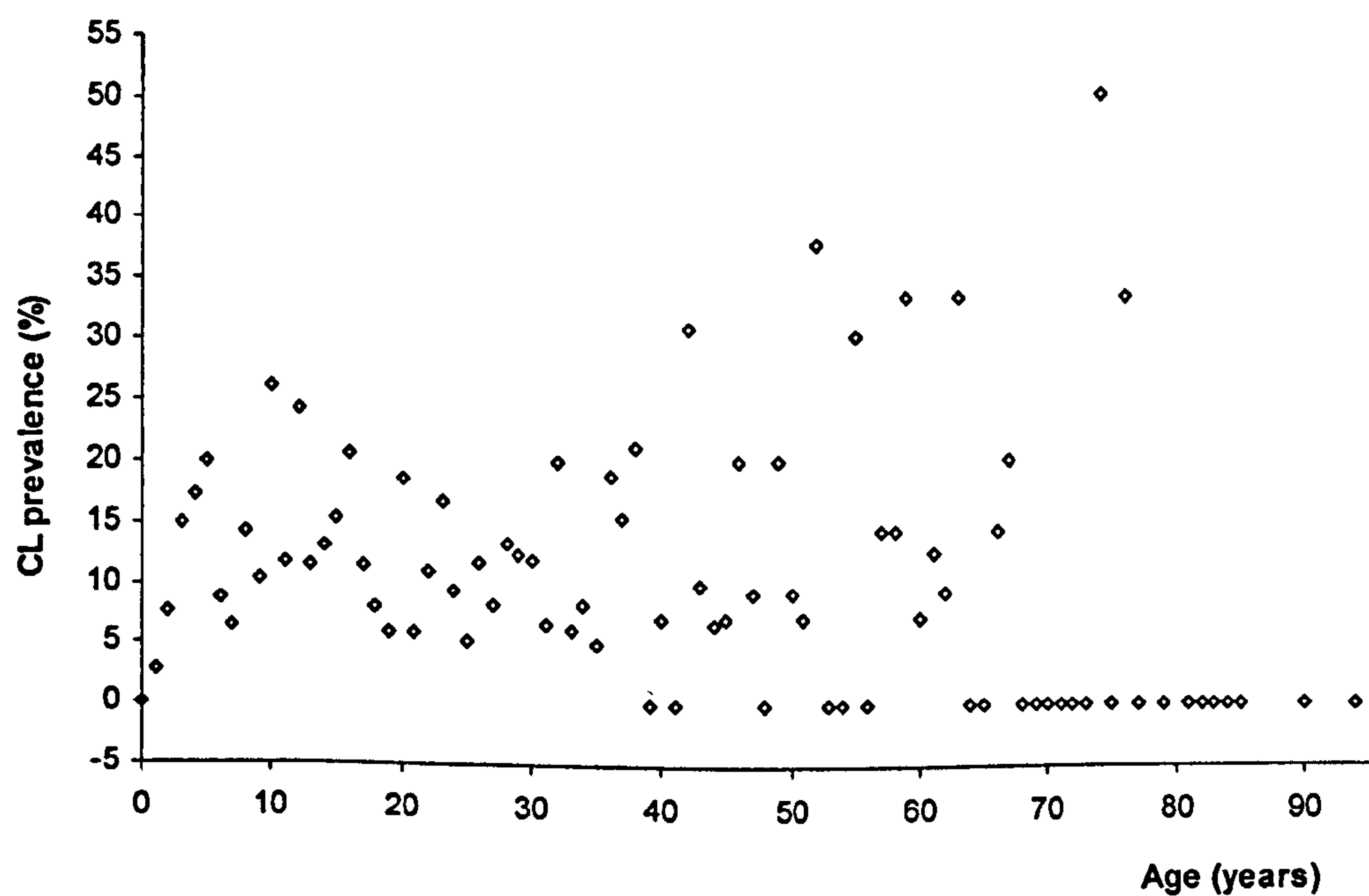
Annexe 38. Indoor abundance of *Lutzomyia nuneztovari* predicted by the MAM against the residuals (Anscombe residuals).



Annexe 39. Observed indoors abundance of *Lutzomyia nuneztovari* (collected with CDC light traps) against their predicted value according to the MAM incorporating village, type of ceiling, number of dogs and pigs within 200 m, number of persons per house, and number of banana plants as explanatory variables.



Annexe 40. Cumulative prevalence of cutaneous leishmaniasis by age. Each point is the proportion with scars or lesions within a one year age band. Note that the number of replicates per age band decreases with age.



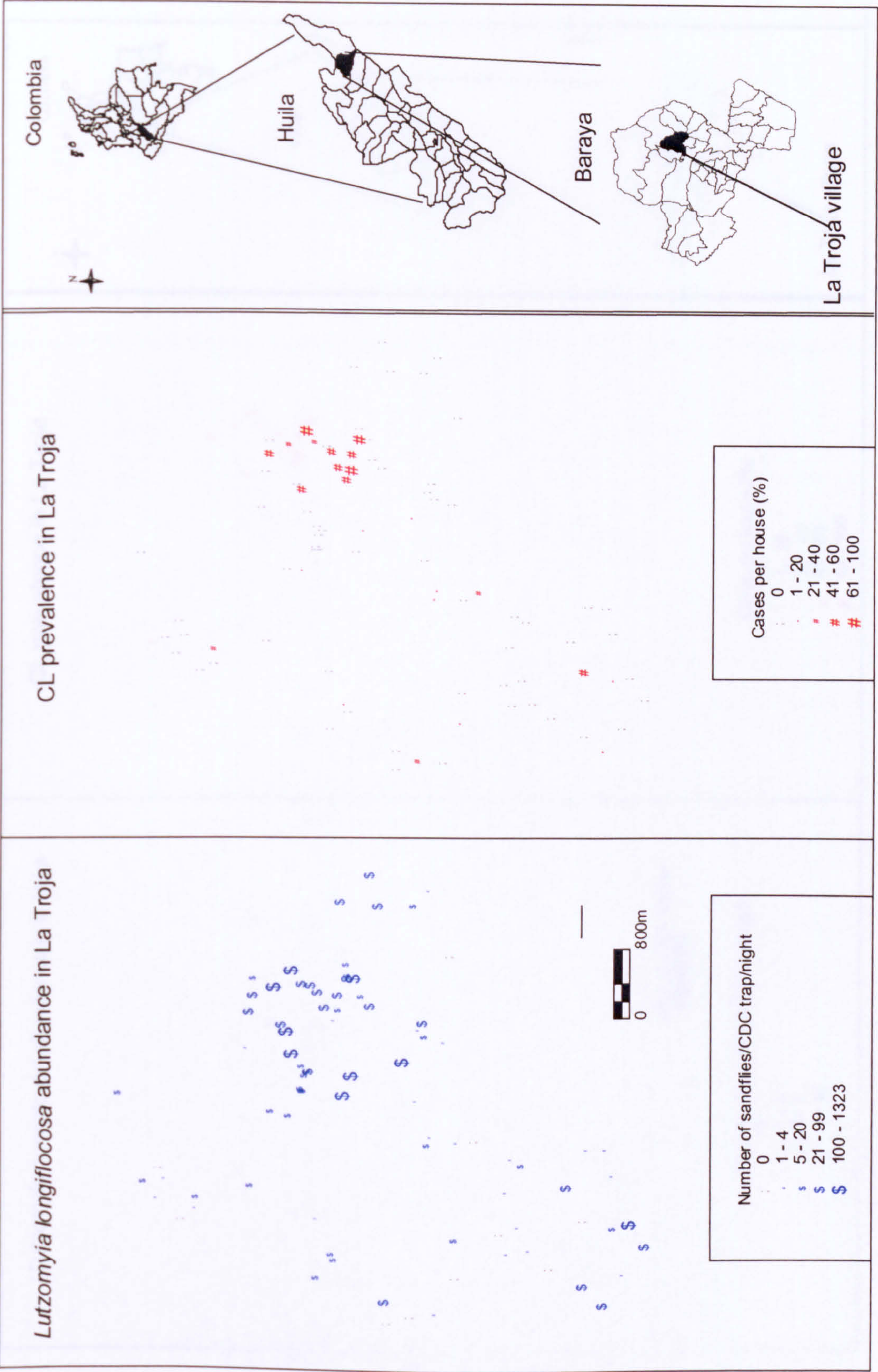
Annexe 41 Significance and explanatory power (r^2 , in percentage) of risk factors for cutaneous leishmaniasis identified by logistic regression analysis.

Varname	X^2	(df)	p	r^2
Altitude	49.95	6	<0.001	5.0
Village	12.28	2	0.002	1.2
Demographic features				
length of residence	19.89	1	<0.001	2.0
Gender	4.55	1	0.033	0.5
House features				
House type	7.64	1	0.006	0.8
Total				10.3

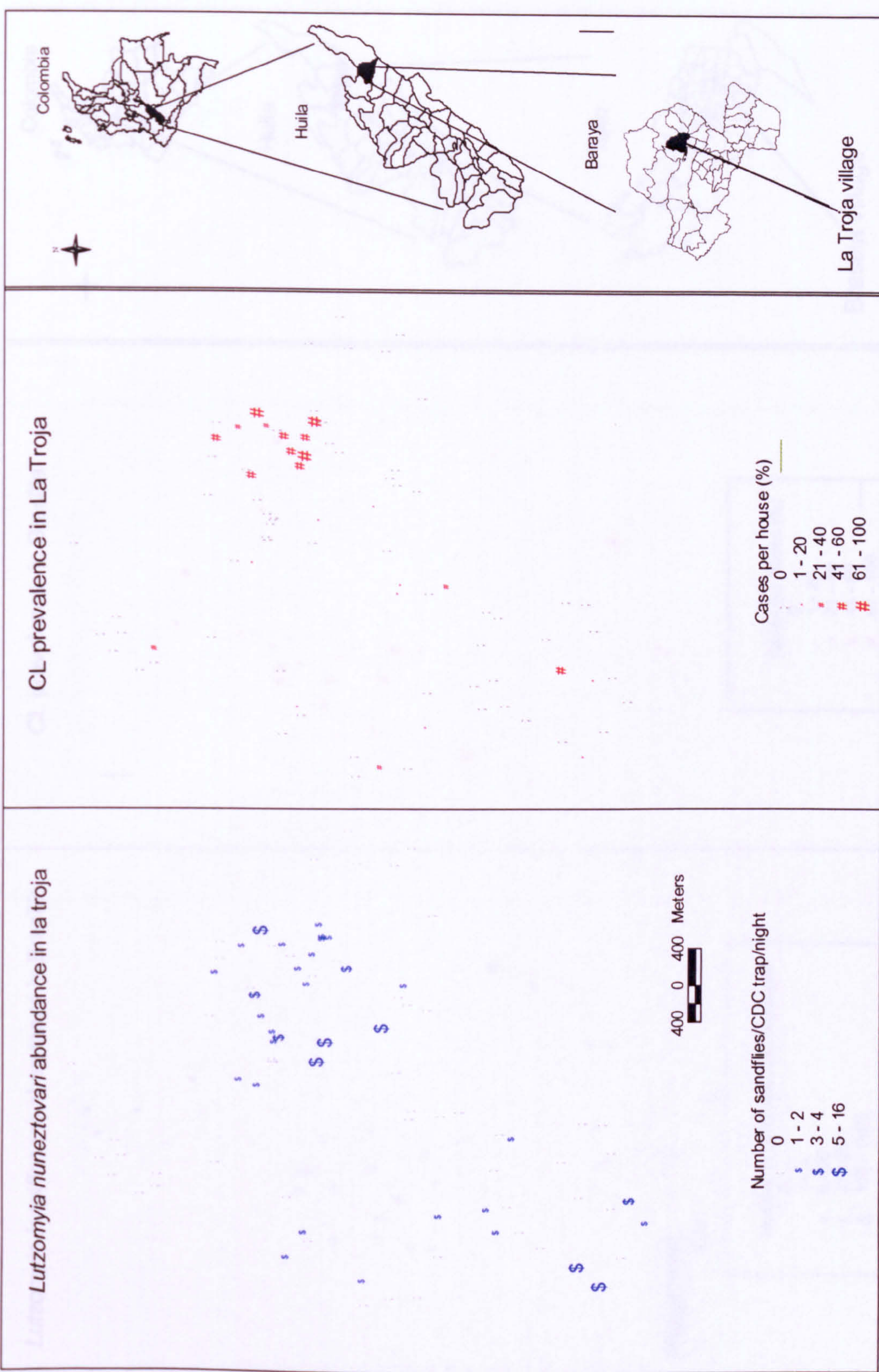
Annexe 42. Significance and explanatory power (r^2 , in percentage) of risk factors for cutaneous leishmaniasis, identified by logistic regression analysis incorporating sandfly abundance.

Varname	X^2	(df)	p	r^2
Altitude	54.69	6	<0.001	5.5
Village	13.28	2	0.001	1.3
Demographic features				
length of residence	22.64	1	<0.001	2.3
Gender	3.58	1	0.059	0.4
Sandfly abundance				
<i>L. longiflocosa</i> females	19.45	1	<0.001	1.9
<i>L. nuneztovari</i> females	12.95	1	<0.001	1.3
Total				14.4

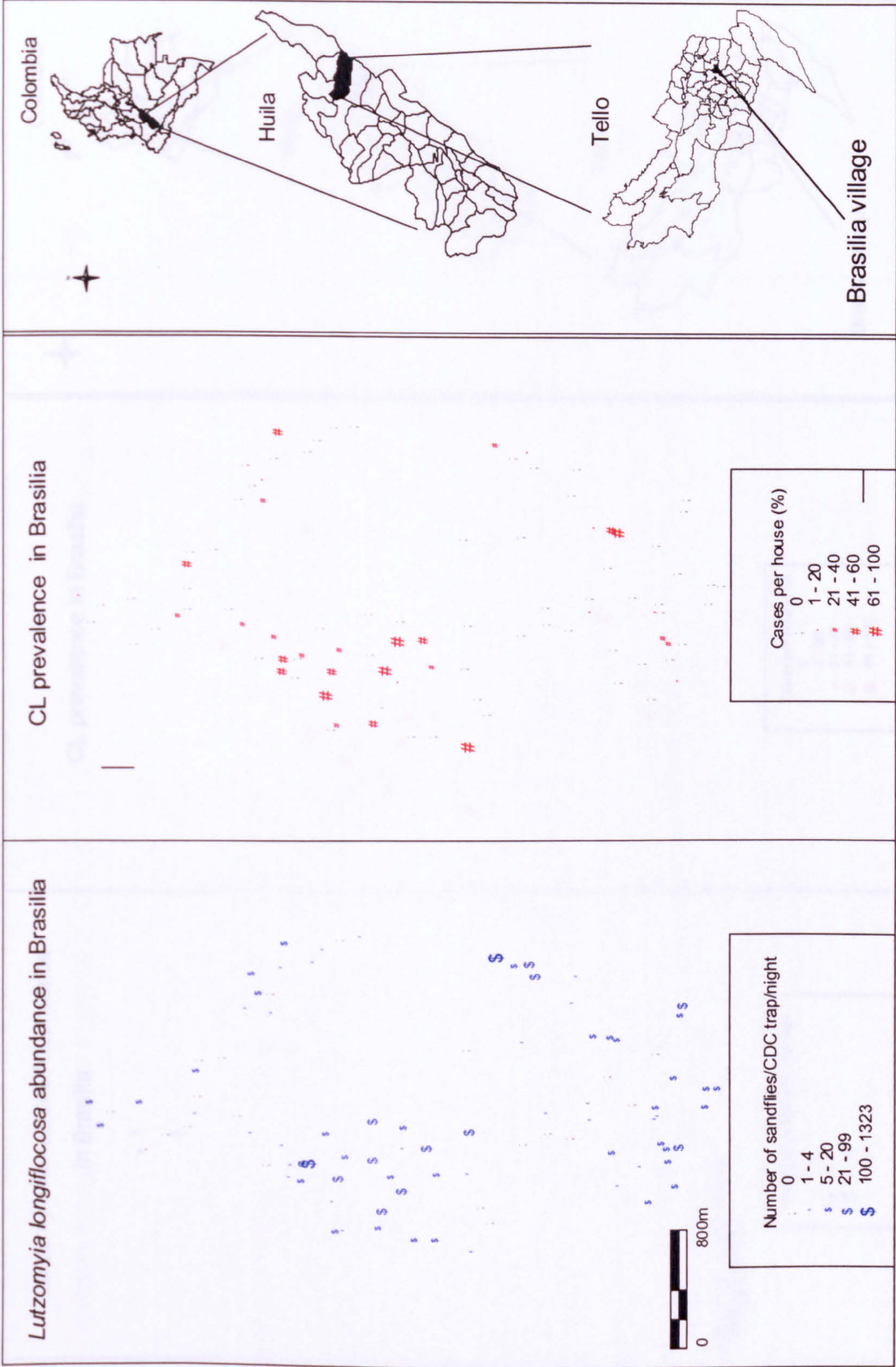
Annexe 43. Spatial distribution of *Lutzomyia longiflocosa* abundance indoors and cutaneous leishmaniasis prevalence (CL) per house in La Troja village (n = 87).



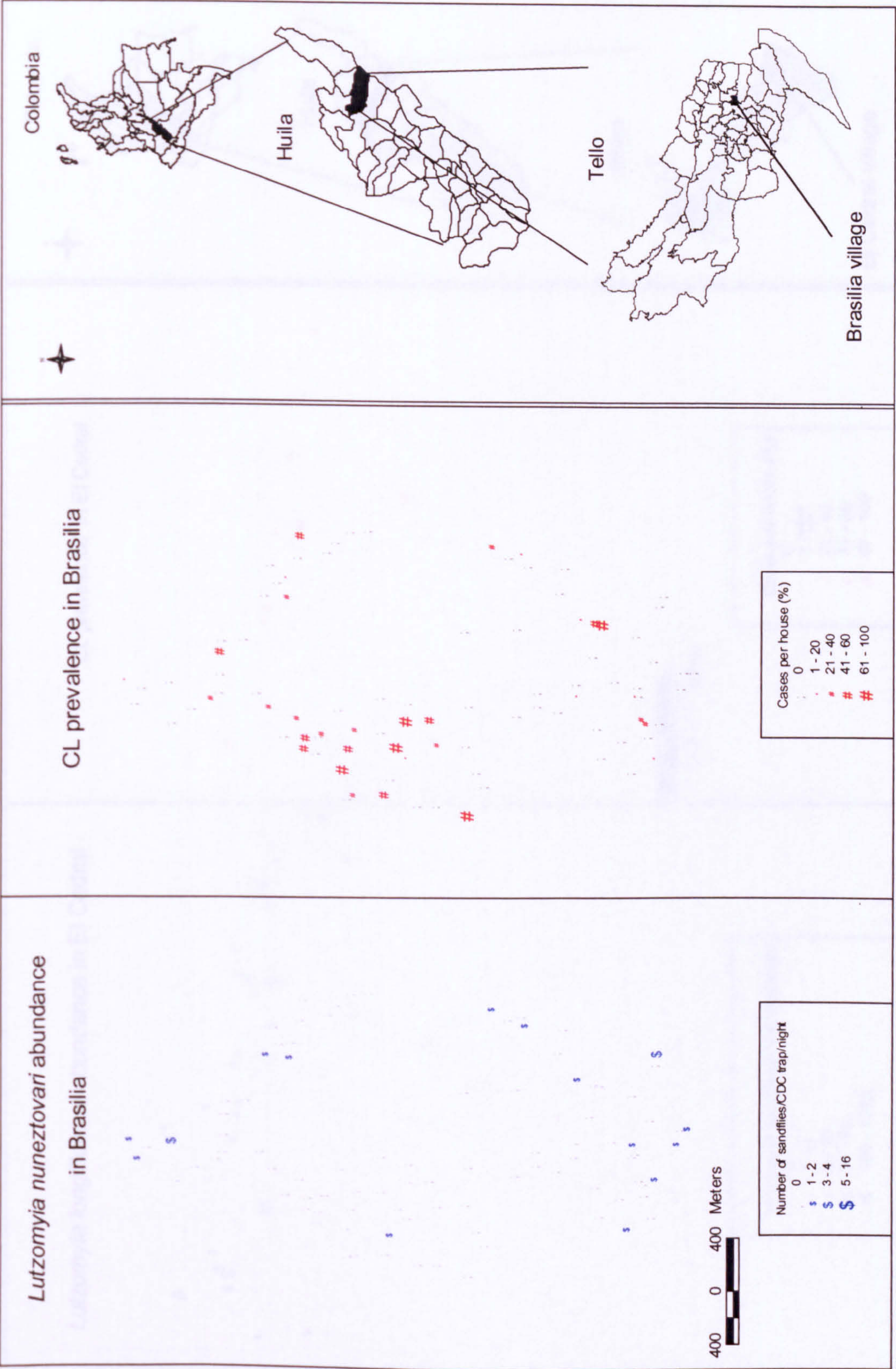
Annexe 44. Spatial distribution of *Lutzomyia nuneztovari* abundance indoors and cutaneous leishmaniasis prevalence (CL) per house in La Troja village (n = 87).



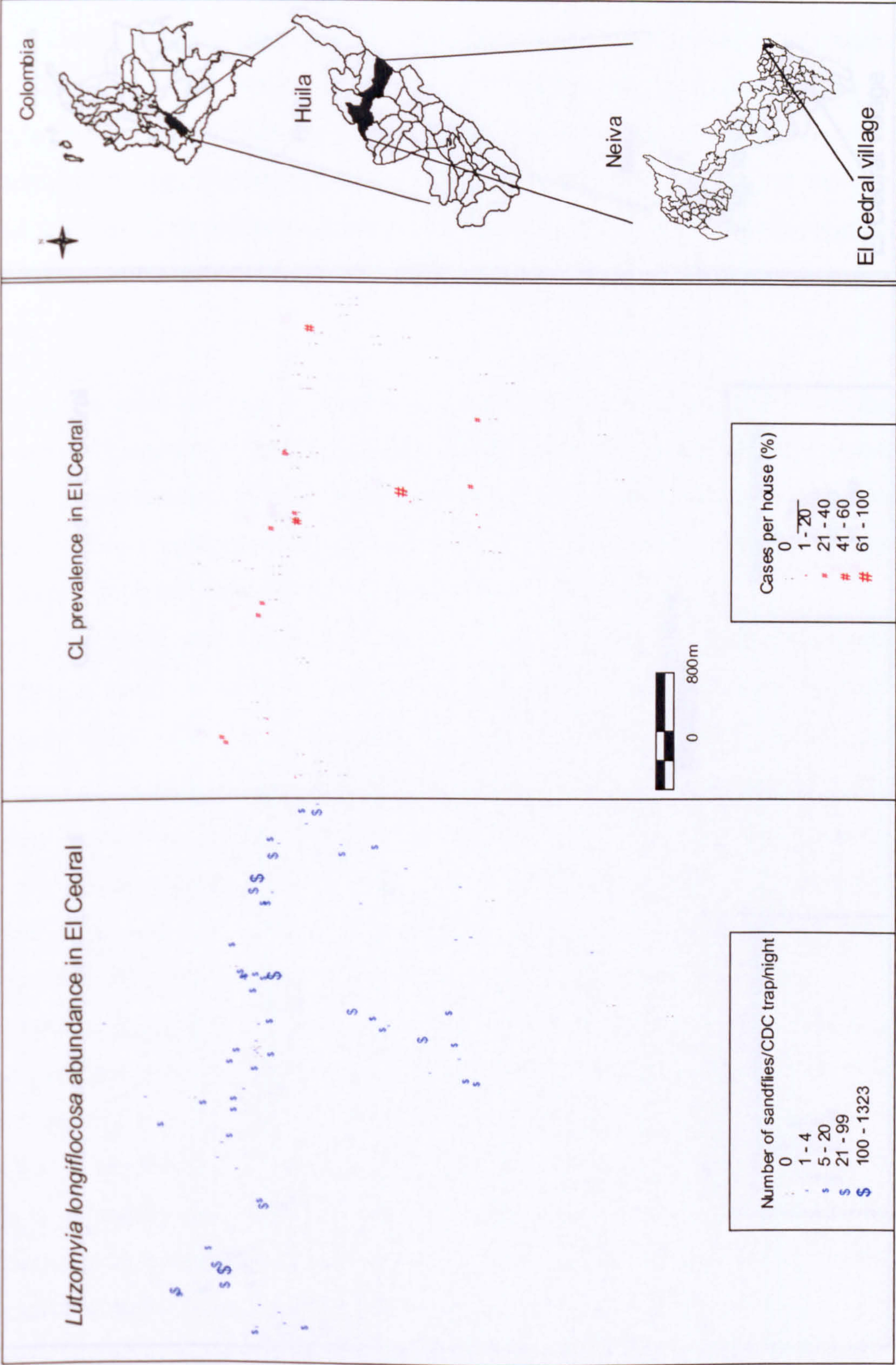
Annexe 45. Spatial distribution of *Lutzomyia longiflocosa* abundance indoors and cutaneous leishmaniasis prevalence (CL) per house in Brasilia village (n = 93).



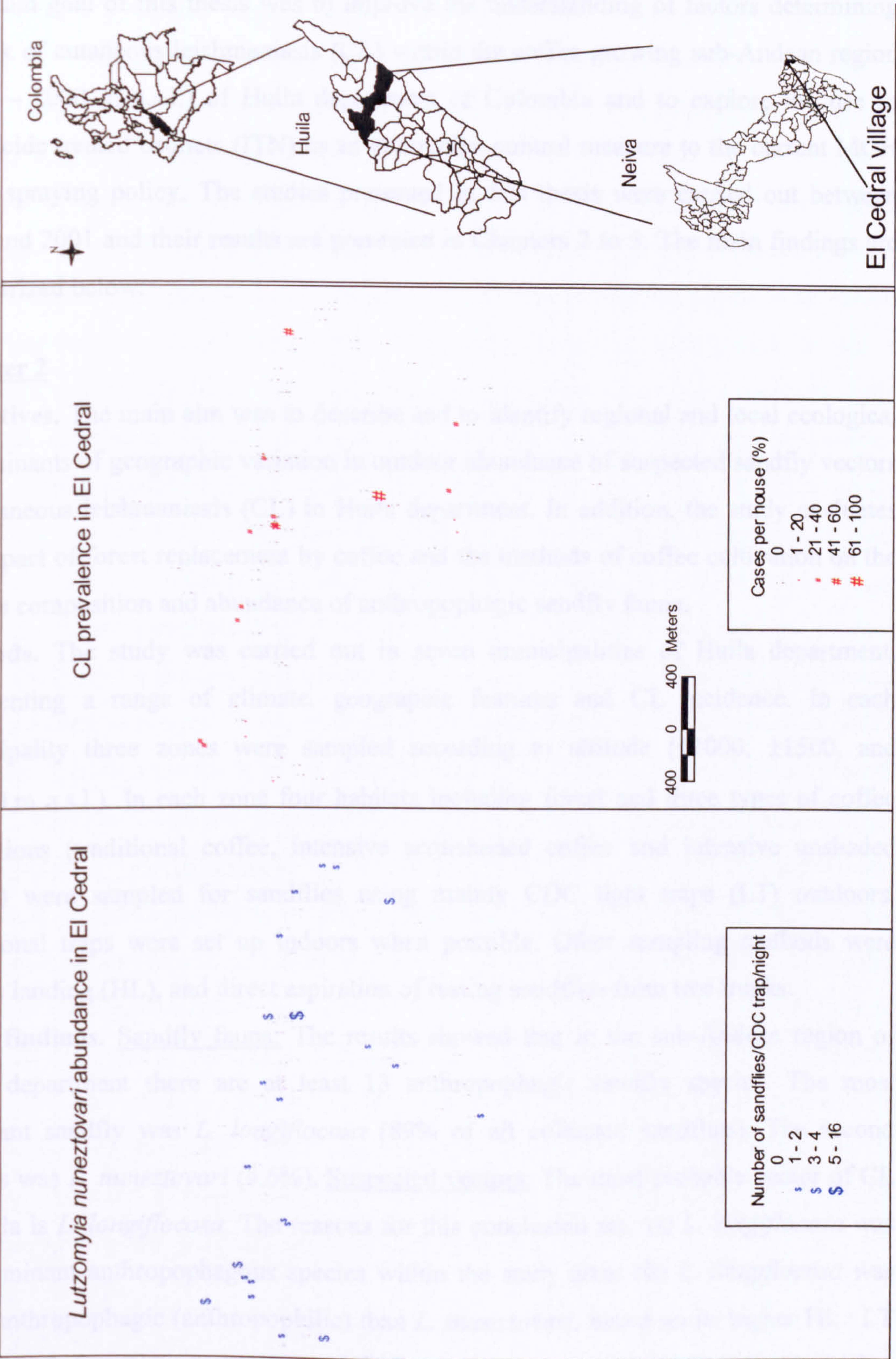
Annexe 46. Spatial distribution of *Lutzomyia nuneztovari* abundance indoors and cutaneous leishmaniasis prevalence (CL) per house in Brasilia village (n = 93).



Annexe 47. Spatial distribution of *Lutzomyia longiflocosa* abundance indoors and cutaneous leishmaniasis prevalence (CL) per house in El Cedral village (n = 85).



Annexe 48. Spatial distribution of *Lutzomyia nuneztovari* abundance indoors and cutaneous leishmaniasis prevalence (CL) per house in El Cedral village (n = 85).



Annexe 49. Executive summary: Main findings of Chapters 2 to 5

The main goal of this thesis was to improve the understanding of factors determining the risk of cutaneous leishmaniasis (CL) within the coffee growing sub-Andean region (1000 – 2000 m a.s.l.) of Huila department of Colombia and to explore the use of insecticide treated bednets (ITN) as an alternative control measure to the current MOH house spraying policy. The studies presented in this thesis were carried out between 1998 and 2001 and their results are presented in Chapters 2 to 5. The main findings are summarized below:

Chapter 2

Objectives. The main aim was to describe and to identify regional and local ecological determinants of geographic variation in outdoor abundance of suspected sandfly vectors of cutaneous leishmaniasis (CL) in Huila department. In addition, the study evaluates the impact of forest replacement by coffee and the methods of coffee cultivation on the species composition and abundance of anthropophagic sandfly fauna.

Methods. The study was carried out in seven municipalities of Huila department, representing a range of climate, geographic features and CL incidence. In each municipality three zones were sampled according to altitude (± 1000 , ± 1500 , and ± 2000 m a.s.l.). In each zone four habitats including forest and three types of coffee plantations (traditional coffee, intensive semishaded coffee and intensive unshaded coffee) were sampled for sandflies using mainly CDC light traps (LT) outdoors. Additional traps were set up indoors when possible. Other sampling methods were human landing (HL), and direct aspiration of resting sandflies from tree trunks.

Main findings. Sandfly fauna: The results showed that in the sub-Andean region of Huila department there are at least 13 anthropophagic sandfly species. The most abundant sandfly was *L. longiflocosa* (89% of all collected sandflies). The second species was *L. nuneztovari* (4.6%). **Suspected vectors:** The most probable vector of CL in Huila is *L. longiflocosa*. The reasons for this conclusion are: (i) *L. longiflocosa* was the dominant anthropophagous species within the study area; (ii) *L. longiflocosa* was more anthropophagic (anthropophilic) than *L. nuneztovari*, based on its higher HL : LT ratio; (iii) *L. longiflocosa* was more endophagic than *L. nuneztovari*, based on its higher LT_{indoors} : LT_{outdoors} ratio; (iv) there was a strong geographic association between the municipalities where *L. longiflocosa* was present and the municipalities where CL has

been reported; whereas *L. nuneztovari* had no apparent association with the presence of CL; and (v) the highest outdoor abundance of *L. longiflocosa* was detected in the municipalities with the highest CL incidence; whereas *L. nuneztovari* was most abundant in municipalities where there were no autochthonous cases of CL. Hence, *L. nuneztovari* seems to play, at best, a secondary vector role. Ecological determinants for the two main suspected vectors: *L. longiflocosa* presented a discontinuous distribution along the sub-Andean region of Huila department with its highest abundance on the West side of the Cordillera Oriental where the foci of CL are located. At local scale, the highest abundance of *L. longiflocosa* was associated with forests which had four tree strata, high cover and high litter cover, and which were located in sites with a relatively high slope and protected from the wind. On a regional scale, preferred sites were located in areas with moderate slope, with mid temperature (18°C - 20°C), rainfall between 1000 - 1800 mm, mid altitude (1500 – 1700 m a.s.l.), and soil of type MQA. *L. nuneztovari* presented a continuous distribution. At local scale, the highest abundance of *L. nuneztovari* was associated with forest or traditional coffee plantations characterized by one tree stratum, with moderate litter cover which had a relatively deep layer of partially decayed litter, located in sites with moderate slope and close to houses. At regional scale, preferred sites were located in areas with a relatively low slope, with a wider temperature range (19°C - 23°C), rainfall between 1600 – 1800 mm , and soil of type MQE. Impact of forest replacement by coffee on anthropophagic sandfly fauna: There was a strong reduction in species richness and abundance of anthropophagic sandfly species from forest to coffee plantations, and there was also an apparent gradient in species richness and abundance from traditional coffee, to semishaded coffee and unshaded coffee. The relatively high abundance of *L. longiflocosa* and *L. nuneztovari* in traditional coffee plantations suggest that these species are “completely adapted” to this type of habitat, which incorporates trees characteristic of the local forest (their pristine habitat). The lower abundance of *L. longiflocosa* and *L. nuneztovari* in the two intensive coffee plantation types indicates that these species are “partially adapted” to these habitats.

Chapter 3

Objectives. The aims were to identify indoor, and around houses, risk factors for CL and its vectors and to provide further incriminatory evidence for the suspected vectors.

Methods. A house based cross sectional study was carried out in three sub-Andean rural areas (La Troja, Baraya municipality; Brasilia, Tello municipality; and El Cedral Neiva municipality) within the epidemic region for CL in Huila department. Information on cases and potential risk factors for CL and sandfly vectors was collected by questionnaires. Sandfly abundance data was recorded by sampling each house with LT.

Main findings. Sandfly risk factors: At least eight sandfly species were caught indoors. The most abundant sandfly was *L. longiflocosa* (93.5% of all collected sandflies). The second species was *L. nuneztovari* (2.1%). Both sandfly species presented an aggregated distribution indicating heterogeneity in human-vector contact. But both sandfly species were found inside a relatively high percentage of houses: 86% with *L. longiflocosa*, and 27% with *L. nuneztovari*. Risk factors detected by multivariate analysis for *L. longiflocosa* were: village (highest in La Troja), altitude (highest between 1600 - 1700 m a.s.l.), percentage of land cover by grass within 300 m of the house (negatively associated), number of houses within 100 m (negatively associated), number of dogs within 200 m (negatively associated), and number of persons per house. Risk factors for *L. nuneztovari* were: village (highest in La Troja), number of banana plants (within 50 m), type of ceiling (highest for close plank), number of dogs within 200 m (negatively associated), number of pigs within 200 m (negatively associated), and number of persons per house.

Risk factors for CL prevalence: A total population of 1427 inhabitants was recorded in 271 sampled houses. Total CL cumulative prevalence was 11.4%, with a significant higher prevalence in males compared with females. Risk factors for CL detected by multivariate analysis were: village (highest in La Troja and Brasilia), altitude (highest between 1600 – 1700 m a.s.l.), gender (highest for male), length of residence in the house, abundance of female *L. longiflocosa*, and abundance of female *L. nuneztovari* (negatively associated).

Vector incrimination evidence: The significant positive relationship between CL prevalence and indoor abundance of *L. longiflocosa* females supports the role of *L. longiflocosa* as the only important vector of CL, at least indoors, in Huila department. This confirms the findings from Chapter 2. The significant negative relationship found between CL prevalence with the abundance of *L. nuneztovari* reinforces the hypothesis that this species has no significant vectorial role in CL transmission in this region. These results provided the rationale for testing an intervention aiming to prevent CL by reducing indoor exposure to *L. longiflocosa* bites, i.e. the use of ITNs.

Chapter 4

Objectives. The studies described in this chapter aimed to evaluate the utility of ITNs, under field conditions, as an alternative to the current Huila MOH policy of house spraying for controlling CL vectors. The following chapter then addresses the social and economic factors which could impact on how easily an ITN programme could be implemented in this region.

Methods. An efficacy trial tested the “potential entomological effect” on *L. longiflocosa* of lambda-cyhalothrin treated bednets as measured by sandfly “indoor abundance”, human landing rates inside and outside bednets, and sandfly mortality rates. The study design was a cross-over in two houses, where one ITN set up in one house was compared with one untreated bednet set up in another house (switched each night). A household effectiveness trial compared the effect of ITNs and house spraying, both with lambda-cyhalothrin, on sandfly “indoor abundance”, blood-feeding success and human blood index (HBI) as detected by indoor LT. The study evaluated three treatments (ITNs, spraying and controls) assigned randomly amongst triplets matched by village and pre-intervention abundance of sandflies. Additionally, validation of LT as representative of indoor HL was tested by comparison with indoor HL catches. Bioassays were carried out to measure the lethal residual effect of the insecticide.

Main findings. Potential entomological effect of ITNs: ITNs reduced human landing rates by *L. longiflocosa* both inside and outside nets. ITNs did not prevent entry but caused immediate mortality for sandflies that had passed through a net. Even untreated wide mesh nets provided some protection. Comparison of ITNs and house spraying: Effectiveness of insecticide on nets persisted for at least 4 months; and “outside-net” landing rates remained significantly reduced in rooms with ITNs 4 months post-treatment. LT catches in rooms with people sleeping under ITNs 4 months post-treatment also contained less sandflies, a lower percentage of bloodfeds, smaller blood meals, and a lower HBI than in control houses. The ratio of LT: HL rates outside ITNs was the same as in control houses demonstrating that the observed reduction in sandfly numbers collected in rooms with ITNs reflects a true difference in risk, presumably because a significant proportion of sandflies get knocked down after contacting a treated net, and before taking a bloodmeal or entering a light trap. The effect of house spraying on sandfly biting rates was more confused. The effectiveness of

insecticide significantly dropped by 4 months, but still caused considerable mortality. LT catches at 4 months found less sandflies, a lower percentage of bloodfeds, smaller bloodmeals, and a lower HBI than in control houses. However, there was evidence that light traps were less effective in sprayed houses as the ratio of LT: HL catches in sprayed houses was considerably less than in control houses. Furthermore, HL catches were not significantly different in sprayed and control houses. Hence, in contrast to the observed ITN effectiveness it is unclear whether individual house spraying (as opposed to mass spraying, which was not tested) protected householders.

Chapter 5

Objectives. To describe household sandfly control practices in a CL endemic area in Huila department, Colombia, and to determine how these are influenced by attitudes, knowledge and socioeconomic status.

Methods. An additional section in the householder questionnaire applied in Chapter 3 collected information on: demography; socioeconomic status; knowledge of cutaneous leishmaniasis, sandflies and their role in transmission; and the control activities practiced. Indoor sandfly abundance data was obtained from the LT catches described in Chapter 3.

Main findings. Amongst 249 interviewees who had lived for at least one year in the sampled houses, 86% knew CL and 98% knew sandflies. Knowledge of the role of sandflies in CL was less widespread, and only 35% of interviewees who knew CL practised measures with the purpose of its control. This practice was higher amongst the 32% who knew that sandflies transmit CL. However, 82% of interviewees practiced sandfly control measures, and these were significantly associated with high sandfly abundance. Control measures included smoke, bednets, and house spraying with insecticide or non-insecticidal substances. Householders using the high cost measures (bednets and insecticide) had the highest economic status. This indicates that household economic status limits the choice of control measure practiced. Health education programmes should note that sandfly nuisance can initiate control measures, but that knowledge of sandflies' role in transmission could enhance activities. The socioeconomic findings indicate that targeted bednet subsidies could reduce inequities in health status amongst CL endemic communities.